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It is characteristic of Science and Progress that they continually open new fields to our vision.—Pearson

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THE BACTERIOLOGY OF THE BUBBLE FOUNTAIN[1]

DOROTHY F. PETTIBONE, FRANKLIN B. BOGART AND PAUL F. CLARK

From the Laboratory of Medical Bacteriology, The University of Wisconsin

The public conscience has been aroused to the dangers of the common drinking cup as a possible means of spreading disease. Many state legislatures have passed laws prohibiting its use; other states have accomplished the same object by rulings of their state boards of health; and interstate passenger trains have been compelled to discard the common cup.

To meet this situation, three substitutes have been recommended; first, the individual cup carried and used by a single person, second, the single service paraffin cup, and third, the bubbling fountain. Expense and inconvenience have been large factors in preventing the general adoption of the first two methods. The bubble fountain, however, has met with cordial appreciation both on the part of the public and also in the hearts of health officials, and has been largely adopted in this country as the modern hygienic method of publicly providing for all comers drinking water free from possible contamination.

But is the bubble fountain as constructed at present free from danger? Have we not seized too readily upon an obvious improvement over the common drinking cup without adequate experimental basis?

Our attention was drawn to the bubble fountain as a possible source of danger during an epidemic of streptococcus tonsilitis which occurred in the fall of 1914 in one of the women's dormitories at the University of Wisconsin. Within a week's time, fifty cases were reported in this building. In studying the situation, it was noted that in this building the water pressure was so low that it was scarcely possible to drink from the bubble foun-

[1] Presented at seventeenth annual meeting of the Society of American Bacteriologists, Urbana, Ill., December 29, 1915.

tains in the hall without touching the metal portions with the lips.

An examination of these fountains showed them to be heavily contaminated with streptococci. Positive results were obtained from the surface of the fountain, from the inside, and from samples of the water. In fact, the swab from the inside of the bubble fountain on the third floor, where the greatest number of cases were located, gave an almost pure culture of streptococci. Several examinations of the city water supplying the fountains showed, on the other hand, no evidence of these organisms. The sanitary character of the city water supply is excellent, the water being obtained from deep wells in the Potsdam sandstone which underlies all southern Wisconsin.

Owing to a laboratory accident the identity of the organisms found in the bubble fountain and those isolated from the cases of tonsilitis was not established; and there were other cases of streptococcus tonsilitis in the town and also scattered cases throughout the University at the time. It seems a fair presumption, nevertheless, that these heavily contaminated fountains were a factor in spreading the infection within the dormitory.

A subsequent investigation of all the other bubble fountains in the University yielded surprising results. In examining the fountains, the following observations were made: type of fountain, height of column, bacterial count of the water on litmus lactose agar and action in glucose broth fermentation tubes which were subsequently examined for streptococci by the hanging drop method and by Gram stained smears. In addition, sterile throat swabs were rubbed thoroughly over the top of the fountain and as far into the bulb as possible. These swabs were placed in glucose broth which also was examined for streptococci by the methods mentioned above. The swabbings proved to be the most fruitful source of positive results. Only in a few instances did the amount of water tested prove to contain streptococci. Forty-three out of seventy-seven bubble fountains (55.8 per cent) were found contaminated with Gram positive streptococci which frequently occurred in long chains.

Many different types of bubble fountains are in use in the

University ranging from a plain rusty half-inch iron pipe with constant flow, in some remote corners of the campus, to the latest porcelain device with automatic shutoff in more prominent situations. In general the simpler fountains such as the rusty pipe were not infected, whereas the complex fountains with intermittent flow and those with crevices and crannies between the inner and outer portions were the worst offenders, although presumably the location of the fountain was a factor in this result. In fact it is worthy of note in connection with the epidemic in the women's dormitory that streptococci were found most often in those buildings frequented by the women students while the Law Building, Engineering Building, Armory, etc., were quite as uniformly free from such contamination.

To control these rather surprising results a second survey of the fountains was made about a year later, at a time when there was no unusual number of upper respiratory infections, but following a period of unusual incidence of "grippe" both in the University and throughout the country. Samples of water and swabbings were taken from fifty fountains, excluding many of those less commonly used. The earlier results were completely confirmed, as Gram positive streptococci were found in 35 out of the 50, 70 per cent of the total.

The streptococci found are somewhat difficult to isolate, and readily overgrown by the common water organisms, developing well in glucose broth but not on other media. They are only slightly virulent to experimental animals. Further studies of these strains are being carried on.

As a further control a Berkefeld filter was attached to a city water faucet and the water allowed to flow for one week. During this period it was estimated that about 3300 liters passed through the filter and yet smears and cultures made from the deposit on the filter showed not only no streptococci but no Gram positive organisms present. This control was repeated.

In order to study the factors involved in the contamination of the fountains, a series of tests were performed on an experimental bubbler in the laboratory. This particular fountain is one of an ordinary continuous flow type, the bubble being fed

through four perforations in the top of a cylinder which screws into the center of a hollow metal bulb. Uniform suspensions of *B. prodigiosus* were made by emulsifying each of two twenty-four hour cultures of the organism in 1 cc. of salt solution. This emulsion (2 cc.) was then dropped slowly from a pipette into the top of the bubble, care being observed not to touch the metal portions of the fountain. Ten cubic centimeter samples of the water were then taken at frequent intervals and plated in triplicate on agar, 1 cc. to each plate. After incubation the plates were allowed to stand at room temperature for several days in order to allow the *B. prodigiosus* colonies to develop their characteristic red pigment. Red colonies and total colonies were then counted. In all cases control samples of the water were taken before introducing the organisms. In a total of over 100 control plates, only one red pigment producer was found. This formed colonies quite different from *B. prodigiosus* colonies macroscopically and could never be mistaken for that organism. Between each two experiments the bubble fountain was removed, washed and sterilized.

The experiments were repeated several times with the column of water at different heights, varying from about 3 to 4 mm. (approximately the height of column prevailing in the women's dormitory at the time of the epidemic) to 10 cm., a column much higher than we have ever observed in use. The results obtained with the column at each level were somewhat variable. So many factors are involved that this is to be expected. The relatively small amount of water sampled as compared with the volume flowing during the period of sampling, the variation in the column caused by variation in water pressure, chance conditions such as the position of the pipettes both in introducing the organisms and in withdrawing the samples, together with unknown factors, must all play a part in the results observed.

In brief, when the bubble is extremely low (3 to 4 mm.) some of the introduced organisms are found in repeated experiments for as long as 135 minutes. With a column 2 to 3 cm. high, the average height of the jets in the University, *B. prodigiosus* organisms are retained frequently for 10 to 20 minutes and have

been found for as long as 90 minutes. A column 7.5 cm. in height has shown the presence of *B. prodigiosus* for 20 minutes although commonly no organisms have been found for more than 2 minutes. Even with the column 10 cm. high, *B. prodigiosus* has always been found in the plates poured one or two minutes after the introduction of the suspension.

Another type of experiment was tried more nearly approximating the ordinary use of the bubble fountain. The writers washed out their mouths with suspensions of *B. prodigiosus* and while the lips were still moist drank from a column 2 to 3 cm. in height, exercising due care not to touch the metal portions of the fountain. Samples of water were taken as before and plated. In one instance the organisms were retained for 135 minutes, and the average was 25 minutes.

The following tables show the results of sample tests taken at random from those made with the experimental fountain:

TABLE 1

Column 2 cm. high. Suspension of B. prodigiosus introduced by pipette, two twenty-four hours cultures 1 cc. salt solution to each culture

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
58	0	Controls
52	0	
56	0	
58	0	5
106	0	10
52	9	15
72	0	20
44	7	25
64	3	30
104	0	40
48	0	50
39	0	60
104	7	70
58	0	80
59	0	90
62	0	100
69	0	110

TABLE 2

Column 2 cm. high. Suspension of B. prodigiosus introduced as in table 1

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
160	0	Controls
130	0	
142	0	
58	13	5
136	4	10
52	1	15
72	13	20
64	2	25
149	21	30
104	35	45
104	5	60
61	1	75
95	2	90
95	0	105

TABLE 3

Column 2 cm. high Suspension of B. prodigiosus introduced as in table 1

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
Not made	0	Controls
	0	
	0	
	5	1
	1	2
	3	3
	0	4
	1	5
	1	7
	1	10
	0	15
	0	20
	0	25
	0	30
	0	40
	0	50
	0	60
	0	70
	0	80
	0	90

TABLE 4

Column 3 cm. high. Suspension of B. prodigiosus introduced as in table 1

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
Not made	0	Controls
	0	
	0	
	7	1
	1	2
	1	3
	1	4
	0	5
	0	7
	193	10
	0	15
	1	20
	0	25
	0	30
	0	40
	0	50
	0	60

These tables show that the efficiency of a bubble fountain depends partly upon the height of the jet thrown. But even at the maximum height used, 4 inches, which is higher than practicable, some organisms are still retained in the column for about 2 minutes. Considering also the fact that when the organisms were introduced on the moist lips, a very small number were added as compared with the millions introduced in the turbid suspension by means of the pipette, it is possible that the saliva of the mouth may be a factor in causing the bacteria to remain a longer time than would otherwise be the case.

How shall we explain these facts and how shall the bubble fountain be made safe for public use?

Our conception of the problem is that the organisms dance in the column of water much as the toy ball dances on top of the fountain in the garden. Most of the organisms introduced are

TABLE 5

Column 10 cm. high. Suspension of B. prodigiosus introduced as in table 1

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
12	0	Controls
20	0	
18	0	
20	0	
14	0	
18	0	
15	2	1
12	1	2
8	0	3
10	0	4
6	0	6
12	0	8
9	0	10
15	0	15
18	0	20
8	0	30

TABLE 6

Column 2 cm. high. B. prodigiosus introduced by rinsing mouth with suspension and then drinking while lips were still moist.

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS IN MINUTES
Not made	0	Controls
	0	
	0	
		Drank from fountain
	0	1
	1	2
	1	3
	2	4
	1	5
	0	7
	0	10
	0	15
	1	20
	0	25
	0	30
	0	40
	0	50
	0	60
	0	70
	0	80

flushed off at once, whereas others "dance" for varying periods of time until they finally fall outside the column and disappear in the waste pipe. Doubtless other factors are concerned, such as variation in pressure due to excessive use elsewhere on the same line and subsequent cessation of this unusual consumption of water. Indeed a control series of plates of water samples taken at five minute intervals from a bubble fountain shows wide variation in bacterial count due partly to variation in pressure. When the pressure is suddenly increased the pipes are vigorously flushed and the bacterial count is higher. Keith[2] (2) has suggested a modified form of fountain which automatically regu-

[2] Keith, S. C., Jr. Amer. Jour. of Public Hygiene, 1910, **20**, 163.

lates the flow so that regardless of pressure changes, the height of the jet will remain constant.

But why retain a vertical column of water when it is obviously the upward direction of flow which more than any other factor renders the bubble fountain unsafe? A fountain (see fig. 1) was constructed with a simple tube, having no crevices or complications placed at an angle of about 55 degrees from the vertical.[3]

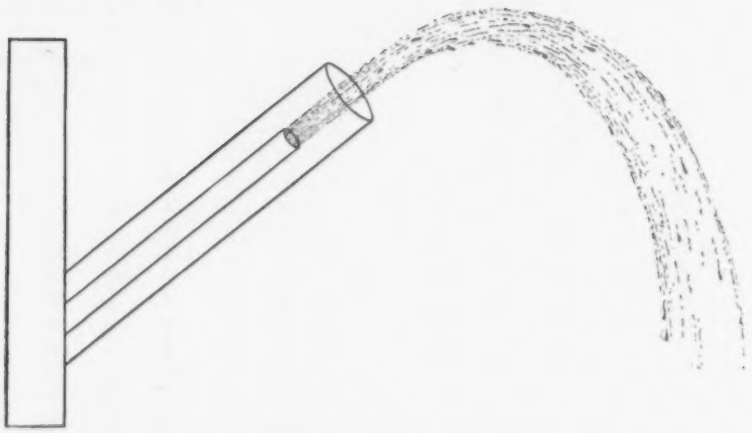

FIG. 1. SIMPLE TYPE OF FOUNTAIN WHICH SHOULD BE GENERALLY ADOPTED. NO CONTAMINATING ORGANISMS ARE RETAINED IN THE JET OF WATER

Even with a minimal stream flowing from this type of fountain, the introduced organisms were all immediately washed away and never did we find a single *B. prodigiosus* colony even on the plates poured immediately after the introduction of the organisms.

This simple type of fountain with an adequate collar guard to prevent possible contact with the orifice would, we believe, solve the problem and furnish water uncontaminated by human lips even to a constant succession of thirsty persons.

[3] Later experiments have shown that a tube erected at an angle of 15 degrees from the vertical is adequate to prevent organisms from "dancing" on the column of water. This makes the construction of a safe bubble fountain much more practicable.

TABLE 7

New model fountain with a small stream of water issuing from a simple tube at an angle of 50 degrees from the vertical. Suspension B. prodigiosus introduced as in table 1

BACTERIAL COUNT	B. PRODIGIOSUS COUNT	TIME AFTER INTRODUCING ORGANISMS
Not made	0	Controls
	0	
	0	
		seconds
	0	2
	0	5
	0	10
	0	15
	0	20
	0	25
	0	30
	0	35
	0	40
	0	45
	0	50
	0	55
	0	60
		minutes
	0	1
	0	2
	0	3
	0	4
	0	5
	0	7
	0	10
	0	15
	0	20
	0	25
	0	30
	0	40
	0	50
	0	60

CONCLUSIONS

1. During an epidemic of streptococcus tonsilitis in a woman's dormitory at the University of Wisconsin, streptococci were found in the bubble fountains in this building and in the water issuing from these fountains.

2. The city water supply was at the time, and has been, excellent in its sanitary character. It is obtained from the underlying Potsdam sandstone. No streptococci were found on a Berkefeld filter through which water had been flowing continuously for one week.

3. Presumably the bubble fountains were a factor in transmitting the disease.

4. A survey of all the fountains of the University showed the presence of streptococci in over 50 per cent of the total number. The streptococci varied in abundance from a few chains to an almost pure culture obtained by swabbings from the fountains in the women's dormitory.

5. In an experimental bubble fountain, *B. prodigiosus* when introduced either by means of a pipette or by the moistened lips remained in the water from 2 to 135 minutes depending partly on the height of the "bubble."

6. Most of the organisms are flushed away but some remain dancing in the column much as a ball dances on the garden fountain even though the bubble be increased to the impractical height of 4 inches.

7. To avoid the difficulty always present in the vertical column, a simple fountain with a tube at an angle of 50 degrees from the vertical was constructed. *B. prodigiosus* was never found in the plates from this type of fountain even when samples were taken immediately after the introduction of the organisms.

8. We believe that this type of fountain should be generally adopted. Its simplicity, low cost of construction and freedom from lurking danger should recommend it to all.

THE ADVANTAGES OF A CARBOHYDRATE MEDIUM IN THE ROUTINE BACTERIAL EXAMINATION OF MILK

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INTRODUCTION

The object of this paper is to direct the attention of laboratory workers to the advantages over plain agar of an agar containing a fermentable carbohydrate, for use in the bacteriological control of market milk. Some laboratories are probably aware of these facts, but since the standard methods for the bacterial analysis of milk prepared by the committee of the American Public Health Association[1] call for the use of plain agar, and since this medium is used in many of the largest laboratories making routine analyses at the present time,[2] it seems pertinent to submit a few data on these points.

METHODS

The bacterial counts reported herein were made on plain agar prepared according to the standard formula, and on lactose agar with the same ingredients plus 1 per cent of lactose. Liebig's beef extract and Witte's peptone were used. The media were adjusted to a reaction of 0.5 per cent normal acid to phenolphthalein, which reaction is, according to the results obtained in this laboratory,[3] better than a higher acidity.

In the determination of bacterial numbers triplicate plates were made of each dilution, and the counts were made after

[1] Meetings of Am. Pub. Health Assn., Rochester, N. Y., September, 1915.
[2] U. S. Pub. Health Service, Public Health Reports, **30**: 2349–2394.
[3] Penn. Agr. Expt. Sta., Report for 1914–1915.

forty-eight hours incubation at 37°C. A hand glass with a magnification of three and one-half diameters was used in counting the plates made with plain agar, but the lactose agar plates were counted with the naked eye.

For the comparison of the size of colonies which developed on plain and lactose agars the plates were incubated forty-eight hours at 37°C. and measurements made by means of a compound microscope and eyepiece micrometer which gave a magnification of fifty diameters.

QUANTITATIVE BACTERIAL ANALYSIS

In table 1 are given the results obtained from eighteen samples of raw market milk when plated on plain and on lactose agars. Among these milks were samples representing all kinds of milk from a very good to a very low-grade type.

TABLE 1

Number of bacteria on plain and lactose agars

Raw Milk

SAMPLE NUMBER	NUMBER OF BACTERIA PER CUBIC CENTIMETER		INCREASE IN FAVOR OF LACTOSE AGAR
	Plain agar	Lactose agar	
			per cent
1	817	1,017	25
2	1,340	2,070	55
3	1,630	2,000	23
4	3,230	3,530	9
5	7,330	7,330	0
6	8,000	11,300	41
7	8,900	12,100	36
8	9,030	8,970	−1
9	11,800	11,170	−5
10	186,000	610,000	228
11	192,000	278,000	45
12	260,000	361,000	39
13	369,000	463,000	26
14	53,700,000	109,000,000	103
15	216,300,000	346,600,000	60
16	421,300,000	640,000,000	52
17	576,000,000	1,216,000,000	111
18	1,036,000,000	1,312,000,000	27

The data given in this table indicate that lactose agar is of considerably more value than plain agar for the quantitative bacterial analysis of milk. Of the eighteen samples examined, fifteen gave higher counts on lactose agar; with one sample there was no difference, while two samples gave counts which showed a negligible advantage in favor of plain agar. If we consider the experimental error as about 20 per cent, and discard all differences of less than that amount, it will be seen that fourteen, or 78 per cent, gave increased counts on the lactose agar, while none of the samples showed a similar increase in favor of plain agar. Taking all eighteen samples we find an average increase of 43 per cent in the counts obtained with lactose agar.

The results reported in table 1 are all from samples of raw milk. In table 2 will be found the data obtained from six samples of pasteurized milk. Three of the samples were pasteurized by the holder method at 140°C. for twenty minutes, while the other three were treated by the flash method at 180°C. The milks were plated within an hour after pasteurization. The results are very striking and demonstrate the superiority of lactose agar over plain agar for the examination of freshly pasteurized milk.

TABLE 2

Number of bacteria on plain and lactose agars

Pasteurized Milk

SAMPLE NUMBER	NUMBER OF BACTERIA PER CUBIC CENTIMETER		INCREASE IN FAVOR OF LACTOSE AGAR
	Plain agar	Lactose agar	
			per cent
1	7,600	25,300	233
2	6,500	42,500	554
3	6,000	69,000	1050
4	18,100	43,600	141
5	23,800	58,000	144
6	72,000	177,000	146

THE SIZE OF COLONIES ON PLAIN AND LACTOSE AGARS

Although the difference in the counts obtained is sufficient reason for advocating the use of a carbohydrate medium for the

bacterial examination of milk, probably the strongest point in its favor is the difference in size of colonies which develop on the two media. The colonies on lactose agar are always considerably larger than those on plain agar, this being especially true of the acid-forming organisms. Photographs were taken of the petri dish cultures obtained and reproductions of some typical plates are appended to this paper. As was stated before, the plain agar plates were counted with the aid of a hand lens as prescribed by the standard methods for the bacterial analysis of milk, whereas the plates containing lactose agar were counted with the naked eye. This practice was followed because the colonies on lactose agar were of sufficient size to make the use of a lens unnecessary. Many trials have failed to reveal colonies on lactose agar under a hand lens which were not visible to the unaided eye. This, however, might not be true in the case of a worker with defective eyesight.

In order to get some definite information on the difference in size of colonies on the two media, pure cultures of *B. lactis-acidi*—which is usually the predominating organism in ordinary market milk—and a streptoccus—which is usually the predominating organism in milk of a certified grade—were plated out on plain and lactose agars, and the colonies which developed were measured after incubation at 37°C. for forty-eight hours. Only plates which contained less than one hundred colonies were used so as to eliminate as far as possible the factor of inhibition. The ten largest colonies on each plate were measured. These data are given in table 3.

These differences in size of colonies are presented graphically in figures 1, 2, and 3.

DISCUSSION

It should be understood that the data here presented are intended to apply only to the routine examination of milk for which a short period of incubation is used. Although the few data at hand indicate that quite similar results are obtained with lower temperatures and longer periods of incubation, it is not within the province of this paper to draw such conclusions.

TABLE 3

Size of colonies on plain and lactose agars

NUMBER OF COLONY	DIAMETER OF COLONIES IN MICRONS					
	B. lactis-acidi (Culture A)		*B. lactis-acidi* (Culture B)		Streptococcus	
	Plain	Lactose	Plain	Lactose	Plain	Lactose
1	50	660	340	560	220	460
2	80	720	280	600	230	440
3	60	540	320	700	220	500
4	50	520	260	1300	220	480
5	60	500	240	600	240	510
6	70	640	220	640	210	580
7	80	820	300	590	240	600
8	60	540	300	1140	220	570
9	60	620	300	1080	260	580
10	70	580	280	1120	220	640
Average.....	64	614	284	833	228	536

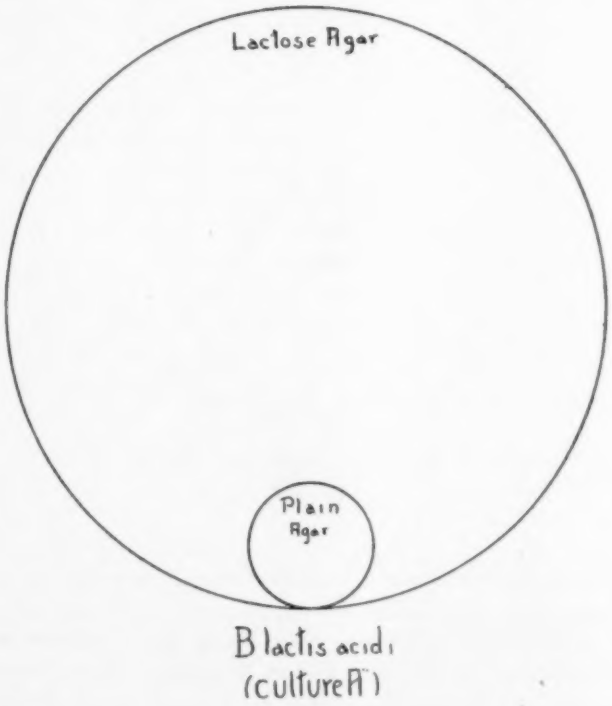


FIG. 1. The relative size of colonies of *B. lactis-acidi* grown on plain and lactose agars. Plates were incubated at 37°C. for forty-eight hours. Graphs are plotted from the average of ten colonies from each medium.

Lactose agar was used in all of the tests herein reported, but from the results of a number of comparisons it may be said that glucose is apparently just as efficient as is lactose, both as to number and size of colonies. According to the work of Heinemann and Glenn[4] glucose and lactose agars are of equal value

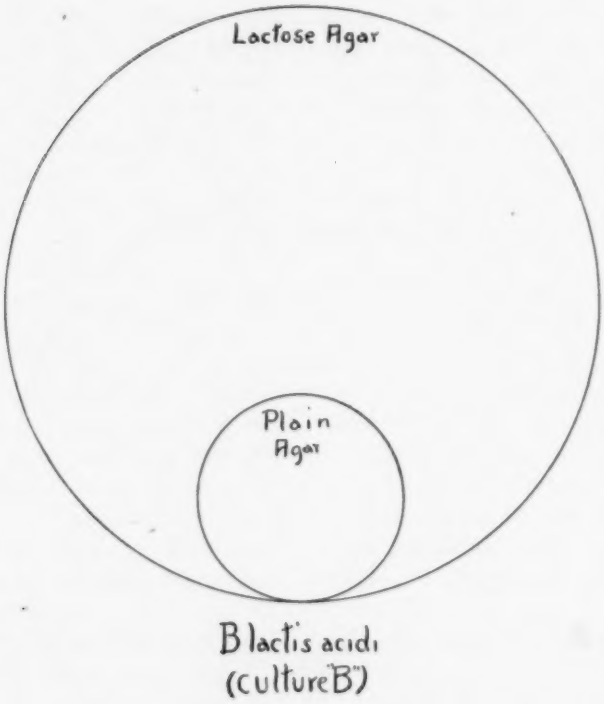


FIG. 2. Same as figure 1 except a different strain of *B. lactis-acidi* was used.

for the quantitative bacterial analysis of milk, and since glucose is cheaper it should, perhaps, be recommended as the standard for routine work. A limited number of trials have also

[4] Jour. Infect. Dis., **5**: 412–420.

indicated that the amount of carbohydrate may be reduced to 0.1 per cent without impairing the value of the medium.

Another advantage of a medium containing a carbohydrate over plain agar is that the former is of considerable value in differentiating the types of organisms on the plates. The colonies of acid-producing bacteria on sugar agar are surrounded by

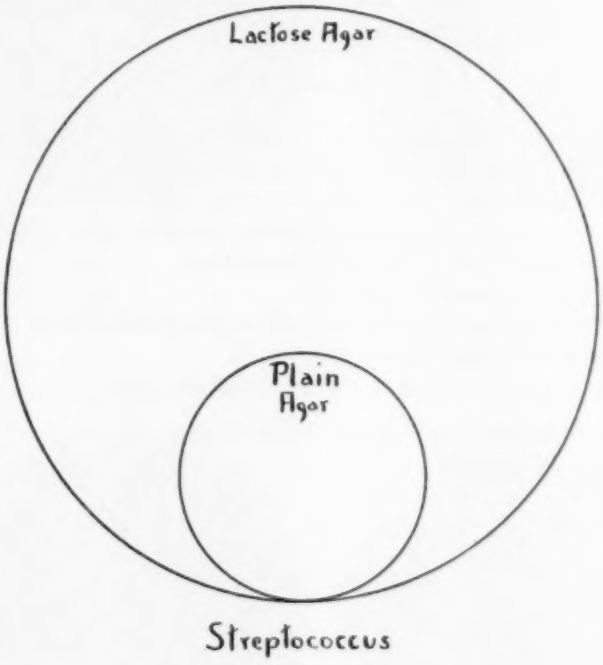


FIG. 3. Same as figure 1 except a culture of a streptococcus was used.

a precipitate of protein matter, thus giving the typical hazy edge which is so characteristic of organisms of the *B. lactis-acidi* group. On agar made without clarification with egg albumen, and which is consequently more opaque, the peptonizing bacteria may be quite readily identified by means of the clear

halo which surrounds their colonies. This differentiation is much clearer on plates containing lactose or glucose agar than on those with plain agar, as the opacity of the medium is very much increased in the presence of a fermentable carbohydrate, due to the growth of acid-producing organisms. In fact, unclarified lactose agar gives, in our hands, nearly as clear a differentiation of bacterial types as does the special casein agar devised by Ayers.[5] These points are well illustrated by the following photographs.

SUMMARY

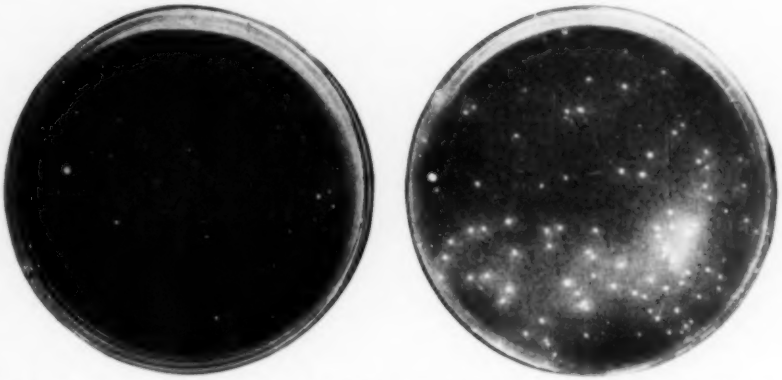
Data are presented which it is thought warrant the recommendation that agar containing glucose or lactose be adopted as the standard medium for the routine bacteriological analysis of milk.

The advantages of a carbohydrate medium over plain agar may be summed up in the following points:

1. A higher count is obtained.
2. The colonies grow larger and more rapidly which greatly facilitates the counting of plates.
3. It is of some value as a differential medium.

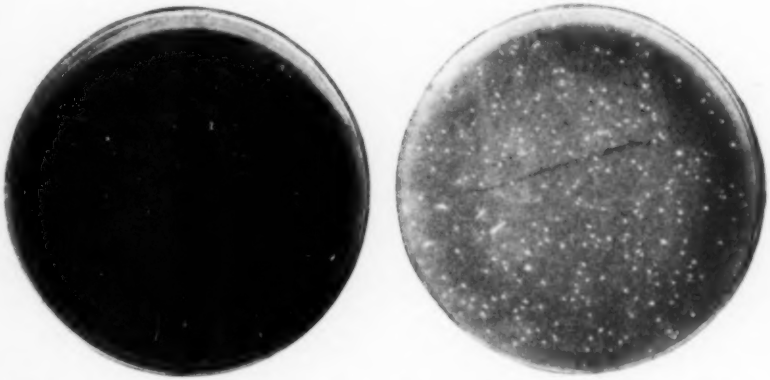
[5] U. S. Bureau of Animal Industry, Report for 1911.

PLAIN AGAR LACTOSE AGAR



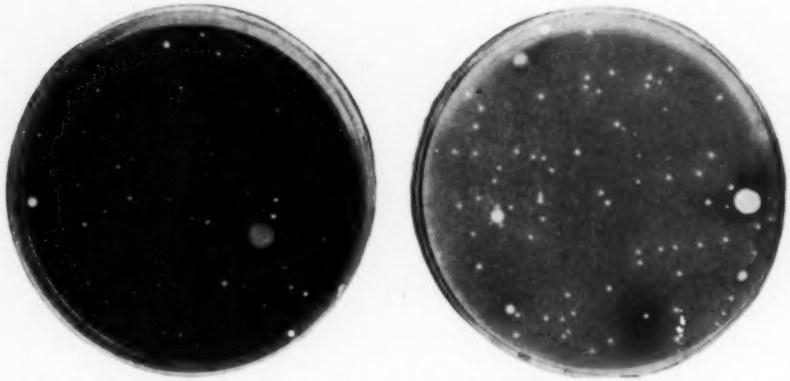
Milk "a." Dilution of 1/100 on plain and lactose agars. Incubated at 37°C. for forty-eight hours.

PLAIN AGAR LACTOSE AGAR



Milk "b." Dilution of 1/1000 on plain and lactose agars. Incubated at 37°C. for forty-eight hours.

PLAIN AGAR LACTOSE AGAR



Milk "c." Dilution of 1/100 on plain and lactose agars. Incubated at 30°C. for six days.

PLAIN AGAR LACTOSE AGAR

Milk "d." Dilution of 1/10,000 on plain and lactose agars. Incubated at 37°C. for twenty-four hours.

ON A SPECIES OF TREPONEMA FOUND IN RABBITS

HANS ZINSSER AND J. G. HOPKINS

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In the course of many attempts to isolate new strains of *Treponema pallidum* from luetic rabbits, the writers have encountered a spiral microörganism which has appeared on six different occasions in tubes prepared from the tissues of five different strains of rabbit syphilis. On first observation of this microorganism it seemed difficult to decide whether it represented a distinct form, or a morphological mutation of *Treponema pallidum*. Subsequent studies, however, have led us to feel reasonably sure that the organism has no relation to *T. pallidum*, but represents a distinct species present in the rabbit tissue, which grows in the tubes as prepared by the Noguchi method. It is a curious fact that although innumerable tubes have been prepared by the same method, in the course of the last three years, with normal rabbit kidney for transplantation of pure cultures of *Treponema pallidum* (both Noguchi's strains and our own), the microörganism concerning which we wish to report has never appeared in such cultures but has been seen only in those implanted with the syphilitic testicular tissue of diseased rabbits. We therefore believe it at least very unlikely that the organism had its origin in the normal rabbit kidney tissue which was used in both kinds of culture. Since we have not made more than a few dozen plants with normal rabbit testis, we can not decide whether the microorganism we are describing may be a parasite in normal rabbit testes, or whether its presence is incidental only to the abnormal conditions occurring in the luetic lesions. To determine this would probably require a great deal of experimentation and time (to say nothing of luck), since we have encountered the organism but six times in the course of three years during which many hundreds of tubes were planted and examined.

It was startling to find this organism growing only when syphilitic material had been planted, and then multiplying with sparseness and cultivated with difficulties much greater than those attending the eventual cultivation of the limited number of strains of *T. pallidum* successfully grown by various workers. However, the obvious suspicions as to its connection with the syphilitic lesions aroused by these, facts seemed easily refuted by the morphology of this treponema which is very different from that of both the virulent treponemata and of the cultivated *T. pallidum* described by Mühlens, Noguchi, and others, and studied for several years by us.

The microörganism is a very fine spiral with curves having the absolute regularity of a corkscrew in most of the individuals, with finely tapering ends, and varying in length from 2 to 10 or 20 rather shallow curves. Both short and long forms are from two to three times as thick as the *Treponema pallidum*, and in most individuals a definite double contour is visible. The average length varies from about one-half to three or four times that of the *Treponema pallidum* and occasional long forms are seen which extend completely across the dark field of a one-twelfth oil immersion lens. The curves are long and shallow.

What is most noticeable about these treponemata is their absolute rigidity and lack of any kind of motility. At first we took it for granted that the organisms were dead. However, when we found that subsequent generations in culture were equally immobile, it became evident that this was characteristic of the species.

The organism stains with great difficulty. Its contours appear faint and are often less distinct than those of the *Treponema pallidum* after 12 hours staining in dilute Giemsa. It can be demonstrated with the Loeffler flagella stain and by the Fontana method. We have so far failed to stain it with the ordinary dyes.

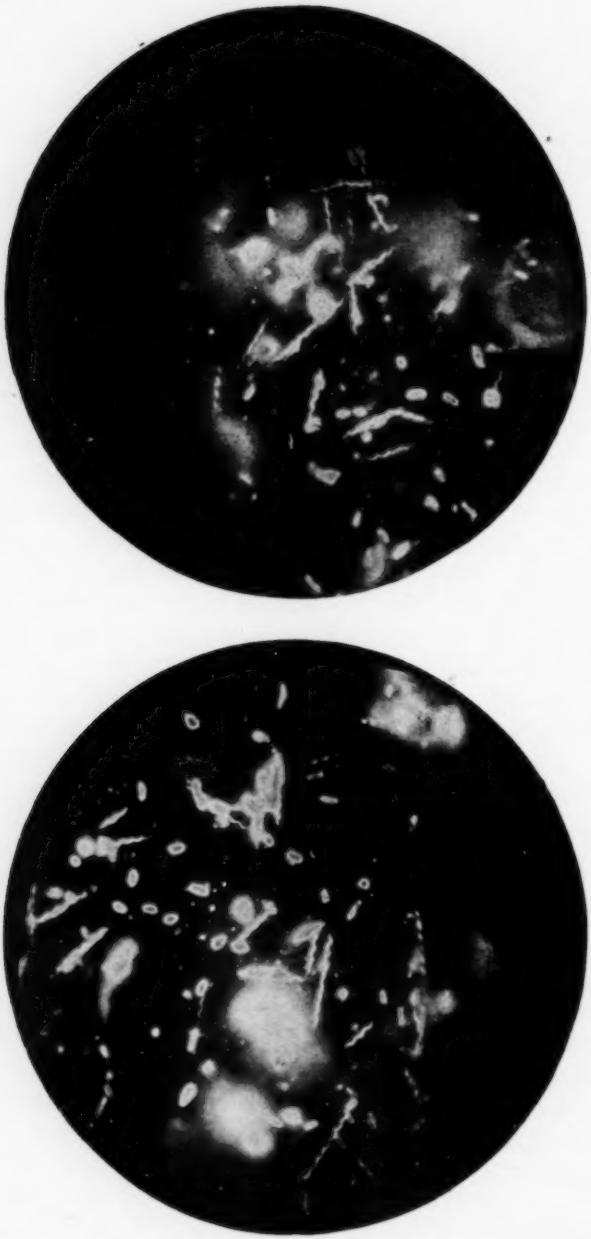
Cultivation is extremely difficult. As stated above, multiplication has appeared in ascitic agar tubes prepared with rabbits' kidney as in the Noguchi method. We have had three or four strains which have proceeded to the third or fourth generation only to be lost. At present we have a strain still growing

in the fourth generation. The cultures in which it multiplied were sealed with oil, and at first we found the organism near the bottom of the tubes, about the kidney tissue. Subsequent study, however, revealed that the most likely place to find them was about one inch from the top under the oil. In such tubes the growth was sometimes indicated by a very faint haziness not seen without careful examination and usually not larger than the head of a pin.

In the material fished from such an area, small clumps of three, four, or five spirals may be seen under the dark field as depicted in the photographs. We have not yet been able to purify this organism, a task of great difficulty because of its immobility. We have not had a sufficiently profuse growth to attain success by the dilution methods, and the first appearance of the microörganism has so far occurred only in contaminated tubes together with motile bacilli. The continued cultivation of the microörganism has, as it were, hung on a thread, the growth being very sparse and taking about a month or two to develop at all.

We have withheld the report on this organism for many months because we have been unable to obtain it in pure culture. Unless chance favors our efforts more than heretofore, it may take a year or two to achieve this, and perhaps we may lose and recover it several times before this result is obtained. We venture to make this brief report now, therefore, since many investigators are studying the cultivation of *Treponema pallidum* from rabbits and we have not seen any mention of a microörganism of this kind, so easily differentiated morphologically from *T. pallidum*, yet appearing in rabbits in the same location and under the same conditions.

Knowledge of its occurrence and its limitation, so far in our experience, to cultures from syphilitic testis should be of value to others working in this field. Should it eventually prove to be a hitherto undescribed species, we would suggest as a suitable name for it "*Treponema rigidum*."



DARK FIELD PHOTO-MICROGRAPHS OF NON-MOTILE TREPONEMA FOUND IN TESTES OF ENETIC RABBITS

STUDIES ON AEROBIC SPORE-BEARING NON-PATHOGENIC BACTERIA

PART II

From the Laboratory of Hygiene and Bacteriology, Johns Hopkins University

SPORE-BEARING BACTERIA IN DUST

BY C. A. LAUBACH

Spore-bearing organisms from dust were obtained by rubbing moist sterile swabs over various dust-laden surfaces, transferring the material thus obtained to melted agar and then heating to 90°C. for fifteen minutes to destroy all non-sporulating bacteria. Plates were then poured in the usual way and different colonies selected for study and identification. In many instances the cultures had to be replated a number of times before the purity of the strain was established, so closely do the spores adhere to each other. In general the most prolific source of the spore-bearing organisms was dust which had lain undisturbed for long periods of time as in closets or on high shelves. Dust particles circulating in the air seemed relatively free from spore-bearing bacteria but an increase of these species was always noted with an increased velocity of the wind. Dust from moist surfaces allowed to dry down and from surfaces exposed to direct sunlight contained few spore-bearers. Numerous strains were obtained from the dust found on books. Some 312 cultures were studied and the types (as established in accord with results of the previous work on milk and on miscellaneous cultures) were found to be distributed as follows.

Bacillus cereus Frankland	93
Bacillus subtilis (Ehrenberg) Cohn	71
Bacillus vulgatus (Flügge) Trevisan	46
(*Bacillus mesentericus vulgatus* Flügge.)	
Bacillus megatherium De Bary	39

Bacillus petasites Gottheil	21
Bacillus mesentericus (Flügge) Migula	17
(*Bacillus mesentericus fuscus* Flügge.)	
Bacillus aterrimus Lehmann & Neumann	8
(*Bacillus mesentericus niger* Lunt.)	
Bacillus fusiformis Gottheil	4
Bacillus brevis Migula	2
Bacillus albolactus Migula	1
Bacillus terminalis Migula	1

In addition to these previously established types, on four occasions an organism was encountered giving the same reactions as the species discovered by Prausnitz in Flügge's laboratory and described by Flügge (1886) as *Bacillus ramosus liquefaciens*. This organism was correctly named *Bacillus prausnitzii* by Trevisan (1889). It is distinct from *Bacillus mycoides* of Flügge but the use of the term, "Würzelbacillus" and the name "Ramosus" for this latter organism by both Eisenberg (1891) and the Franklands (1894) with a coincident description of *Bacillus mycoides* as a distinct species by the latter has led to hopeless confusion. Our investigations show that the majority of organisms of this group, producing felted growths in the depths of agar, correspond in all particulars to *Bacillus mycoides* of Flügge which is probably identical with the "Würzelbacillus" and also with the *Bacillus ramosus* of both Eisenberg and the Franklands. The *Bacillus ramosus liquefaciens* of Flügge is a distinct species which we shall describe under its correct name *Bacillus prausnitzii* Trevisan (syn. *Bacillus ramosus liquefaciens* Flügge).

Two cultures were isolated which exhibited the morphology and general cultural characters of the members of the "mesentericus" group but produced an abundant yellow pigment. At first we were inclined to regard this organism as identical with the species described by Sternberg (1892) as *Bacillus subtilis similis*, but the morphology was so clearly that of the mesentericus type that it was deemed best to describe it as a new variety of *Bacillus mesentericus* to which the varietal name *flavus* is given. On one occasion a culture was obtained which seems to represent *Bacillus ruminatus* of Gottheil. It shows the peculiar porcelain-white growth on agar said by Gottheil to be the prin-

cipal feature distinguishing *B. ruminatus* from *Bacillus megatherium*, which it most closely resembles. The species described originally by Vogel (1897) as *Bacillus mesentericus panis viscosi I*, now known as *Bacillus panis* Migula was found once; and one isolation proved to be a new species belonging to the mycoides group to which we have given the name BACILLUS ADHAERENS. The above list of organisms found in dust must therefore be supplemented by the following types whose descriptions are given below in full.

Bacillus prausnitzii Trevisan	4
(*Bacillus ramosus liquefaciens* Flügge.)	
Bacillus mesentericus variety *flavus* nov. var.	2
Bacillus ruminatus Gottheil	1
Bacillus panis Migula	1
(*Bacillus mesentericus panis viscosi* I Vogel.)	
BACILLUS ADHAERENS, nov. sp.	1

Bacillus prausnitzii Trevisan

This organism was originally described as *Bacillus ramosus liquefaciens* by Flügge. It is sometimes regarded as identical with *Bacillus mycoides* but a culture obtained from the Kral collection in Vienna shows different reactions from those of *Bacillus mycoides*. Several cultures corresponding closely to the Kral culture were isolated from dust. The following description applies to the Kral culture and to our own isolations, as well.

Morphology. In young cultures 6 hours old on plain agar the organisms are homogeneous, have round ends when free and flattened ends when in juxtaposition. They generally occur in chains of 2 to 4 elements and resemble *Bacillus mycoides* in morphology. The single cells measure 0.625 to 0.75 by 3 to 5 microns. On glucose agar they are thicker measuring 0.75 to 1 by 3 to 5 microns. In older cultures, 24 hours, on both plain and glucose agar the protoplasm is converted into globular bodies which take the stain badly. These are especially abundant on glucose agar which also shows long and thick vegetative rods measuring 0.75 to 1 by 4 to 6 microns and peculiar washed-

out organisms which seem to be made up of a fine network or skein of filaments. (Figures 53 and 54.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form early appearing in 24 hours on plain and on glucose agar. They are usually central, one to a cell, and are slightly wider than the vegetative rods. The organisms with spores retain their chain formation and later the free spores may also remain attached in chains. The free spores are cylindrical and measure 0.75 to 1 by 1.5 to 2 microns. As they lose their protoplasm they become oval and measure 0.625 to 0.75 by 1 to 1.25 microns.

Agar slant. Profuse spreading dull growth consisting of fine interlacing filaments developing from the central line of inoculation as a rhizoid mass. The early growth is extremely tenacious and extends deeply into the underlying agar. Later the growth becomes finely granular and friable and can be scraped from the medium. In general the appearance on agar is like that of a culture of *Bacillus mycoides.*

Agar stab. Abundant growth along line of inoculation and spreading surface growth.

Agar colonies. Colonies consist of profusely interlacing filaments spreading from opaque centers. They are dull grayish and penetrate the agar, under the surface of which they grow in the medium.

Glucose litmus agar slant. Scanty growth on the surface with a pronounced acid reaction which remains permanent.

Glucose litmus agar colonies. Colonies much the same as those on plain agar but somewhat more profuse, with the filamentous character more pronounced.

Gelatin stab. Progressive funnel-like liquefaction often complete within three days.

Gelatin colonies. Colonies consist of profusely interlacing filaments spreading from opaque centers. They are dull greyish and penetrate the gelatin, under the surface of which they grow as in agar. Each colony is soon surrounded by a zone of liquid gelatin.

Broth. Granular scum and flocculent growth which soon settles to the bottom.

Peptone. Similar granular scum and flocculent sediment.

Potato. Viscous yellowish-gray growth spreading profusely and rapidly over the whole medium.

Litmus milk. Reaction highly acid within 24 hours and a firm coagulation within 48 hours. Peptonization soon begins and proceeds very slowly eventually converting the coagulum to an amber-colored fluid. Coagulation is more rapid in freshly isolated strains.

Blood serum. Profuse moist dull interlacing or mycelioid growth. No peptonization.

Fermentation tubes. Glucose: a flocculent growth in the bowl and a granular scum. Turbidity in closed arm. Reaction acid.

Saccharose: a flocculent growth in the bowl with an acid reaction. Arm usually clear.

Lactose: a similar flocculent growth with an acid reaction. Arm usually clear.

Thermal death point. Spores survive 12 pounds in the autoclave but are killed by 15 pounds. In the Arnold they survive 45 minutes but are destroyed by an hour's exposure.

Bacillus mesentericus variety *flavus* nov. var.

This is a new variety of *Bacillus mesentericus* to which the name *flavus* is given because of the abundant yellow pigment it produces. We have encountered it repeatedly in dust and in soil.

Morphology. Thin homogeneous rods with round ends, in young cultures on plain agar measuring 0.375 to 0.5 by 1.5 to 4 microns. On glucose agar the organisms are a little thicker and longer, measuring 0.5 to 0.75 by 3 to 5 microns. They often grow in long threads measuring 9 to 12 microns in length. Shadow forms are formed early both on plain and on glucose agar. In old cultures especially on glucose agar the long forms tend to curve. (Figures 55, 56 and 57.)

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form within 24 hours. By the end of the 2nd day the spores are abundant. They arise in the center or towards one end of the organism and bulge the rod but little. The free spores retain rims of protoplasm often greater in quantity at one end than at the other. They resemble the mesentericus spores and measure usually 0.625 by 0.75 to 1.5 microns. As they mature they lose their rims of protoplasm, become more oval and measure 0.5 to 0.625 by 0.75 to 0.875 microns.

Agar slant. Moist, smooth, non-spreading lemon-yellow growth. The lemon-yellow color becomes more pronounced with age.

Agar stab. Profuse granular growth along line of stab with slight irregular outgrowths.

Agar colonies. The colonies are smooth, moist, round, with no tendency to spread, glistening and raised, lemon-yellow in color. There is a definite opaque yellow center while the periphery is translucent and shell-like. Deep colonies have a tendency to spread and become faintly iridescent.

Glucose litmus agar slant. A very scant, moist, granular and slightly yellow growth is produced with a slight acid reaction.

Glucose litmus agar colonies. Colonies similar to those on agar but less profuse. Reaction of medium acid.

Gelatin stab. A very slow liquefaction is produced along the line of stab and a slight yellow tinge is imparted to the medium.

Gelatin colonies. The colonies rest in slight depressions caused by slow liquefaction. They are dark yellow in color, moist, round and smooth. Under the low power of the microscope each colony is seen to be composed of concentric circles of varying densities with lobate edges.

Broth. A slight turbidity appears after a considerable period. The medium clears by sedimentation and the sediment has a slight yellow tinge.

Peptone. A slight turbidity, somewhat less than in broth occurs. This soon settles to the bottom.

Potato. No visible growth.

Litmus milk. No change in reaction even after a long period of time.

Blood serum. A scant, moist, smooth, glistening and yellowish growth is produced. No solution of the medium occurs.

Fermentation tubes. Glucose: a slight turbid growth occurs in the bowl with the production of a slight acidity.

Saccharose: the same appearance. No acid.

Lactose: slight turbidity. No acid.

Thermal death point. Spores survive 18 pounds pressure in the autoclave but are destroyed by 20 pounds. They survive one hours steaming in the Arnold.

Bacillus ruminatus Gottheil 1901

The type "ruminatus" was first described by Gottheil (1901). In morphology and cultural characters it closely resembles *Bacillus megatherium* from which it is distinguished by its porcelain-white growth, particularly in young cultures. The present description applies to an organism found in dust and subsequently in water which corresponds to Gottheil's original description but not however to that given by Chester. We believe that it may properly be called *Bacillus ruminatus.*

Morphology. Homogeneous rods with rounded ends measuring 0.625 to 0.75 by 2.5 to 4 microns in young cultures on plain agar. On glucose agar they are distinctly thicker and longer measuring 0.75 to 1.125 by 2.25 to 5 microns. Rarely, long forms are found on this medium measuring 8 to 10 microns in length. Shadow or washed out forms are common on both plain and glucose agar measuring 1.025 to 1.5 by 3 to 5 microns. Organisms often appear in short chains. (Figures 58 and 59.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early, often in 48 hours, especially when the strains are first isolated. They are central or slightly ex-centric and on sporulation swell or bulge the organisms from which they spring. They measure usually 0.625 by 1.25 to 1.5 microns. Some spores are however somewhat smaller and more oval.

Agar slant. Moderate growth along line of inoculation forming a definite ridge. There is a tendency for the growth to spread to either side and the opacity of the growth decreases towards the periphery which is translucent. The growth is glistening, raised, moist and has a pure white color which becomes more apparent with age.

Agar stab. Growth along the line of inoculation distinctly granular.

Agar colonies. The colonies are very characteristic. Some are round and regular while others show a considerable tendency to spread. They are moist, opaque, raised, glistening and white. The majority show dense centers surrounded by thin areas which are in turn surrounded by translucent shell-like peripheries.

Glucose litmus agar slant. The growth is similar to that on agar although somewhat variable as to amount. The distinct white coloration is also evident. A definite and permanent acidity is produced.

Glucose litmus agar colonies. The colonies correspond closely to those on agar but are less profuse.

Gelatin stab. A fairly rapid progressive cone-like liquefaction occurs.

Gelatin colonies. The colonies rest in a cup-like excavation caused by liquefaction. They are definitely circumscribed and have an opaque center which is surrounded by a less dense grayish area. This in turn is enclosed by a more dense grayish ring. Outside this ring the opacity decreases towards the periphery.

Broth. A fine fragile pellicle is formed with some turbidity. The medium clears by sedimentation.

Peptone. Similar scum but less marked turbidity.

Potato. Cream-white moist profuse growth developing in 24 to 48 hours.

Litmus milk. Within 24 hours a slight acid reaction occurs and the milk often shows a distinct thickening at the bottom. The coagulation is not definite however and peptonization begins usually within 48 hours. As it advances a clear zone of amber-colored fluid is found at the upper part of the milk tube. This gradually increases until all the milk is peptonized.

Blood serum. Fairly profuse moist glistening smooth whitish growth. Some softening of the serum but no definite liquefaction.

Fermentation tubes. Glucose: a slight turbidity in the bowl and neck with a reduction of the litmus in the closed arm. Reaction acid.

Saccharose: a similar growth with an acid reaction.

Lactose: only a slight turbidity develops with an alkaline reaction.

Thermal death point. The spores survive 20 pounds pressure in the autoclave but are destroyed by 22 pounds. They survive one hours steaming in the Arnold.

Bacillus panis Migula 1900

This organism was originally described by Vogel (1897) as *Bacillus mesentericus panis viscosi I.* It has been found in Baltimore, but once, in dust.

Morphology. When first isolated this organism showed only encapsulated forms, the capsules staining readily with gentian violet. As the organism was cultivated on artificial media in the laboratory it lost its capacity of forming easily-stained capsules but continued to manufacture a quantity of viscous material which gave a characteristic appearance to the cultures. In young cultures on plain agar, 6 to 24 hours old, the organisms are small and homogeneous with round to flattened ends, measuring 0.375 to 0.5 by 1.5 to 3 microns. They show no appreciable difference in thickness on glucose agar, but tend to show long forms measuring 5 to 6 microns in length. Occasional shadow forms are seen measuring 0.75 to 1 by 1.5 to 4 microns. (Figures 60, 61, and 62.)

Motility. No motility has thus far been demonstrated.

Staining properties. Gram-positive.

Spore formation. When first isolated, spores were formed early, often in 24 to 48 hours. After long cultivation in the laboratory, they appear in the cultures only after 6 to 8 days growth. They are formed in the centers or towards one end of the rods and are

typical mesentericus spores. They retain definite rims of protoplasm at times concentrated at one end of the spore and measure 0.375 to 0.5 by 1 to 1.25 microns. As they lose their rims of protoplasm they become more oval and measure about 0.75 by 1 micron.

Agar slant. Growth scanty in 24 hours, then becomes slightly raised, finely wrinkled, translucent, non-spreading and viscous. When older the growth has a tendency to become somewhat dry and gray and is easily scraped off. On highly acid agar the growth is more profuse within 24 hours but is not viscous.

Agar stab. Slight granular growth along the line of inoculation with occasionally a slight budding out from the stab.

Agar colonies. Colonies small, drop-like, slightly irregular, showing little or no tendency to spread, glistening, elevated and viscous. In some cases a scum-like covering which enclosed clear gelatinous material was produced about each colony.

Glucose litmus agar slant. Moderate non-spreading growth within 24 hours. The surface of the growth has a sort of honeycombed appearance caused by fine interlacing wrinkles. It is also viscous and is somewhat bluish-gray in color. The reaction is definitely acid within 24 hours. This acidity is followed by a reduction of the litmus and a gradual return to alkalinity.

Glucose litmus agar colonies. The colonies correspond closely to those on plain agar but usually attain somewhat greater dimensions.

Gelatin stab. Rapid funnel-like and progressive liquefaction. Complete liquefaction results with the formation of a tenacious grayish scum.

Gelatin colonies. The colonies on gelatin plates rest in cup-like excavations caused by rapid liquefaction. A definite brown center with a surrounding grayish granular area is evident in each colony under the low power of the microscope.

Broth. A slight turbidity is produced within 24 hours with the beginning formation of a scum. The scum later is finely granular and is formed of discrete colonies. The medium clears itself by sedimentation.

Peptone. Reaction the same as that in broth.

Potato. Growth finely wrinkled, grayish and viscous. The wrinkles appear wave-like. When older the growth loses its viscosity and becomes dry and granular.

Litmus milk. A gradual clearing from the top due to progressive peptonization occurs within 24 hours. Within 48 hours peptonization is generally completed. The remaining fluid has a port-wine color but becomes amber-colored after a variable period.

Blood serum. Smooth, moist, glistening and viscous growth. Within 24 hours some solution of the medium occurs along the line of inoculation (a trough-like excavation). The medium may be entirely dissolved within 2 weeks, and a tenacious scum may be formed.

Fermentation tubes. Glucose: finely granular scum is formed which generally breaks up into large flakes. A flocculent growth is present in the bowl. The reaction is definitely acid.

Saccharose: the growth is the same and the reaction also acid.

Lactose: the growth is also abundant with more pronounced scum-formation. Reaction alkaline.

Thermal death point. The spores survive 10 pounds in the autoclave but are destroyed by 15 pounds pressure. They survive 30 minutes steaming in the Arnold but are destroyed by one hours exposure.

Bacillus adhaerens nov. sp.

This organism has been encountered but once, in dust. It is apparently a new species.

Morphology. Slender long rods with homogeneous protoplasm and flat ends, growing usually in long curved chains made up of 18 to 20 elements. In young cultures on plain agar the individual cells measure 0.375 to 0.5 by 1.5 to 4 microns. Some longer forms, 6 microns in length, may also be found. On glucose agar the organisms are homogeneous, measure 0.625 to 0.75 by 3 to 5 microns, but are often longer measuring 6 to 8 microns. In older cultures (4 days) many globular bodies occur on glucose agar. They resemble the globular bodies seen in *Bacillus cereus.* (Figures 63 and 64.)

Motility. No motility has ever been observed, even in very young cultures.

Staining properties. Gram-positive.

Spore formation. When first isolated this species formed spores in 24 hours. After long artificial cultivation spores are formed only after 4 to 5 days growth on both plain and glucose agar. They are usually subterminal but may be central. The rods swell appreciably before sporulation, sometimes in the center and sometimes at the ends. The free spores are oval and measure 0.625 to 0.75 by 0.875 to 1 micron. They often remain fastened to each other in long chains. Frequently a bit of protoplasm remains attached to the spore which then resembles a tennis racket with its handle.

Agar slant. In early growth (18 hours) this species slightly resembles *Bacillus mycoides.* The line of inoculation shows a distinct ridge from which shoot out fine interlacing filaments. These adhere closely to and grow into the agar. Considerable puckering of these interlacing filaments causes a roughened leathery appearance on the surface. The early growth is moist and slightly glistening but these properties are soon lost. The edges of the growth are serrated, with little or no tendency to spread. A brownish color is found in old cultures throughout the entire medium.

Agar slant. Profuse growth along the line of inoculation and out into the medium. This has the appearance of an inverted fir tree.

Agar colonies. The colony is very characteristic. It first appears like a small colony of *Bacillus mycoides,* but within 24 hours the filaments seem to swell and produce a somewhat corrugated surface with a very definite, elevated and yellow-brown center. The entire colony adheres closely to the agar and gradually grows into it.

Glucose litmus agar slant. The growth is similar to that on plain agar but is very scanty. A definite and permanent acidity is produced.

Glucose litmus agar colonies. Colonies in this medium are considerably smaller than those on plain agar and have the same general appearance.

Gelatin stab. In gelatin, growth is slow and a very slow funnel-like liquefaction is produced.

Gelatin colonies. They appear coarsely granular, slightly raised with definite yellow-brown centers. The centers are coarsely flocculent under the low power of the microscope. The colonies are surrounded by a slight area of liquefaction.

Broth. A slight turbidity is produced and a definite scum is formed which settles to the bottom.

Peptone. Growth similar to that in broth.

Potato. A fairly profuse grayish-white moist growth. When the medium is dry the growth is scale-like.

Litmus milk. No change is noticed within 24 hours. After 48 hours a slowly progressive peptonization occurs. The medium becomes amber-colored.

Blood serum. A fine-grained leather-like growth occurs, dull gray and adherent to the medium. Later this is easily scraped off. No solution of the serum occurs.

Fermentation tubes. Glucose: a flocculent growth occurs in the bowl and extends into the closed arm. Reaction acid.

Saccharose: turbidity in bowl. Arm clear. Reaction neutral or slightly acid.

Lactose: turbidity in bowl. Arm clear. Reaction not changed.

Thermal death point. The spores resist 18 pounds pressure in the autoclave but are destroyed by 20 pounds. They survive one hour's steaming in the Arnold.

SPORE-BEARING ORGANISMS IN WATER

BY C. A. LAUBACH

The spore-bearing organisms in water were obtained by passing the tap water in the laboratory through Berkefeld filters under pressure for a period of three days, washing the filters in sterile salt solution, heating the washings to 80°C. for 15 minutes and then plating. Ten samples were obtained by this method and 313 organisms studied. The species previously established from studies of milk and dust were found as follows:

Bacillus cereus Frankland	93
Bacillus subtilis (Ehrenberg) Cohn	84
Bacillus vulgatus (Flügge) Migula	45
(*Bacillus mesentericus vulgatus* Flügge.)	
Bacillus mycoides Flügge	41
Bacillus megatherium De Bary	14
Bacillus mesentericus (Flügge) Migula	12
(*Bacillus mesentericus fuscus* Flügge).	
Bacillus petasites Gottheil	8
Bacillus aterrimus Lehmann & Neumann	7
(*Bacillus mesentericus niger* Lunt.)	
Bacillus fusiformis Gottheil	2
Bacillus brevis Migula	2
Bacillus prausnitzii Trevisan	1
(*Bacillus ramosus liquefaciens* Prausnitz.)	
Bacillus ruminatus Gottheil	1

In addition to the above types one organism was found which had not thus far been isolated in our laboratory but which was described originally by Chester as a viscous variety of *Bacillus subtilis*. Another sporulating organism new to us, producing a green pigment, was found on one occasion. An attempt was made to identify this as one of the numerous fluorescent sporulating organisms already described in water but the morphology and cultural reactions were quite the same as those of *Bacillus cereus* and it was therefore decided to regard it as a fluorescent variety of this species. It has subsequently been isolated on a number of occasions. An organism was encountered on one instance which had such peculiar properties as to merit description as a new species. Therefore the following may be added to the above list.

Bacillus subtilis var *viscosus* Chester	1
Bacillus cereus var *fluorescens* nov. var.	1
BACILLUS LATEROSPORUS nov. sp	1

Bacillus subtilis variety *viscosus* Chester 1903

Attention was first called to this organism by Chester (1903). We have encountered it but once, in water.

Morphology. Homogeneous rods with round and flat ends measuring 0.5 to 0.625 by 1.5 to 4 microns in young cultures on plain agar. No change in morphology on glucose agar. Trans-

parent or shadow forms abundant on plain and on glucose agar. These measure usually 0.75 to 1.125 by 2.5 to 4 microns. In old cultures an abundance of viscous substance appears staining slightly with gentian violet. (Figures 65, 66 and 67.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. When first isolated, spores were formed within 48 hours. After long cultivation on artificial media, the spore formation has become considerably delayed. Spores are now formed only in ten to fourteen days. They are central or slightly ex-centric and resemble the spores of *Bacillus subtilis.* They measure usually 0.5 to 0.625 by 1 to 1.125 microns and are thus oval to cylindrical. They do not bulge the organisms when formed.

Agar slant. Within 48 hours there is a fair growth which is moist, glistening, non-spreading, glairy and transparent. Its characteristic quality is its viscosity. Later the growth becomes dull and grayish in its appearance and loses its viscosity. A slight wrinkling appears along the outer edge of the growth and forms a narrow border surrounding the entire line of growth. After a few days the medium acquires a brownish color.

Agar stab. A faint granular growth along the line of inoculation.

Agar colonies. Colonies round, definitely circumscribed, with but little tendency to spread, raised, dull and viscous. There is a scum-like covering which encloses gelatinous material about each colony which is very characteristic.

Glucose litmus agar slant. The growth is bluish-gray, viscous, wrinkled and dull. A slight reduction of the litmus occurs with a definite acidity. There is a later return to alkalinity usually within ten days.

Litmus glucose agar colonies. The colonies are similar to those on plain agar but are more profuse and have a definite bluish-gray color.

Gelatin stab. Rapid progressive surface liquefaction. Complete liquefaction occurs within 96 hours with the formation of a definite flocculent sediment.

Gelatin colonies. The colonies are definitely circumscribed and rest in a cup-like excavation due to liquefaction. They are translucent, grayish, viscous with opaque centers.

Broth. A granular, lace-like scum is formed which settles to the bottom. A flocculent growth occurs throughout the medium.

Peptone. Growth similar to that in broth.

Potato. A profuse, grayish, slightly glistening and viscous growth is produced. More characteristic is the appearance of numerous blebs filled with gelatinous material on the entire medium within 24 hours. Later the growth becomes dry and scale-like.

Litmus milk. Within 24 hours a slight reduction of the litmus. Within 24 hours a thickening occurs at the bottom and peptonization begins. This progresses rapidly and within four days the process is generally completed. There remains first a port-wine, then an amber-colored fluid.

Blood serum. The growth is scant, glistening and bluish-gray. There is occasionally a fine wrinkling. A gutter-like excavation along the line of inoculation is often visible. Within 96 hours a pinkish tinge is produced in a wrinkled scum if water of condensation be present at the bottom of the tube.

Fermentation tubes. Glucose: a turbid growth occurs in the bowl and neck with the formation of a thin smooth bluish-gray scum. Some acidity is produced.

Saccharose: a similar growth is apparent but the scum is finely granular. Here also a definite acidity is produced.

Lactose: the growth is identical with that in saccharose but the reaction remains neutral or becomes alkaline.

Thermal death point. Spores survive 15 pounds in the autoclave but are killed by 20 pounds pressure. They are killed within an hour in the Arnold sterilizer.

Bacillus cereus variety *fluorescens.* nov. var.

An organism producing a greenish fluorescence has been encountered frequently in water and in soil. It does not agree with any previously described organisms in its cultural reactions,

but has the same morphology and in many respects the same reactions on media, as *Bacillus cereus*. It has therefore been described as a fluorescent variety of this organism.

Morphology. In young cultures on plain agar (6 to 20 hours) the vegetative rods have rounded ends, slightly granular protoplasm and measure 0.5 to 0.75 by 1.5 to 4 microns. They frequently grow in short chains. On glucose agar they are slightly thicker and longer measuring 0.75 to 0.875 by 2 to 5 microns. As the organisms mature they show many globular bodies in their protoplasm which take the stain with difficulty. These are particularly abundant on glucose agar. (Figures 68, 69, and 70.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form early appearing in 24 hours on plain and on glucose agar. They are located in the center or towards one end of the vegetative rods which swell noticeably on sporulation. As the spores mature the protoplasm disintegrates but remains attached to the spore, sometimes in equal quantities at the ends and sometimes in greater amount at one end than at the other. The spores thus resemble mesentericus spores but are much larger. The free spores measure 0.5 to 0.75 by 0.75 to 1.5 microns. They frequently remain attached to each other in long chains.

Agar slant. The growth is profuse, smooth, moist, and dull. There is a definite ridge along the line of inoculation and the edges are thin and slightly serrated. A yellow-green pigment is diffused throughout the entire medium. This remains permanently and increases with age. The pigment is especially pronounced in an acid medium. Rarely there is considerable wrinkling and the formation of rather coarse folds.

Agar stab. Slight granular growth along the line of stab with the gradual appearance of the characteristic pigment.

Agar colonies. Irregular colonies which have a great tendency to spread in amoeboid fashion. They are glistening, slightly translucent and slightly raised. They become more dense and dull as growth progresses and assume a greenish-yellow coloration. The colonies can be scraped off and are of a

mealy consistency. The same pigment appears in the medium.

Glucose litmus agar slant. The growth is fairly profuse and similar to that on agar. The reaction is first acid with a return to alkalinity after a considerable period.

Glucose litmus agar colonies. The colonies are similar to those on agar but more profuse. Reaction of medium acid.

Gelatin stab. Very rapid liquefaction along the line of inoculation. The medium is entirely liquefied within 48 hours and acquires an intense yellow-green color. A scum of the same color is formed.

Gelatin colonies. The colonies rest in cup-like excavations caused by liquefaction. They have a definite greenish center surrounded by a net-work of fine interlacing, clear, thread-like filaments. The medium assumes the characteristic fluorescence.

Broth. Profuse, flocculent growth with the production of a slight green-yellow coloration.

Peptone. Growth more pronounced than in broth and the coloration more intense. A scum is also formed.

Potato. Profuse, moist, mealy, yellow-green growth with a coloration of the medium.

Litmus milk. Slight reduction of the litmus with a suggestion of peptonization within 24 hours. A precipitation of the proteins occurs within 48 hours and peptonization progresses rapidly. A scum is formed and the fluid remaining is deeply colored with the characteristic pigment.

Blood serum. Moist, profuse, dull and slightly greenish-yellow growth. No liquefaction.

Fermentation tubes. Glucose: profuse flocculent growth with the formation of yellow-green scum in the bowl and a turbid growth in the closed arm. The usual fluorescence is present. A definite acidity is produced.

Saccharose: the growth is identical with that in glucose but no acidity is produced.

Lactose: the growth is the same. Reaction unchanged.

Thermal death point. The spores survive 12 pounds pressure in the autoclave but are destroyed by 15 pounds. They are also destroyed by one hour's exposure to steam in the Arnold sterilizer.

BACILLUS LATEROSPORUS nov. sp.

This organism was obtained but once, from water. It is apparently a new species.

Morphology. The typical vegetative rods are found only in very young cultures 6 to 8 hours old. They are homogeneous or slightly granular with round ends, measuring 0.375 to 0.5 by 1.125 to 4 microns on plain agar. On glucose agar they are slightly thicker and about the same length measuring 0.5 to 0.75 by 1.125 to 4 microns. The rods rapidly lose their characteristic appearance and assume a fusiform or spindle shape with a swollen middle and pointed ends. Such forms take the stain much less intensely. (Figures 71 and 72.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. As the organisms swell and assume the fusiform shape the spores appear at one side as slightly staining globules. The protoplasm soon disintegrates but more on one side of the spore than on the other so that the free spores which are almost egg-shaped retain a rim of protoplasm on one side and present a characteristic appearance. Such spores measure 0.75 to 0.875 by 1.5 microns. As the spores mature they lose their rims of protoplasm, become more oval and measure 0.75 to 1 by 1 to 1.125 microns. The spores begin to appear in about 48 hours and are abundant in 72 hours. Rarely small tags of protoplasm remain attached at the ends of the spores.

Agar slant. The growth is very characteristic. It is profuse, rapidly spreading, smooth, moist, and has a silvery metallic-like lustre. Within 72 hours the growth becomes slightly wrinkled. With age the growth loses its metallic lustre and becomes dull and scrapes off in scales.

Agar stab. A slight grayish granular growth along the line of stab.

Agar colonies. The colonies are flat, transparent, irregular, moist, and have the same peculiar metallic lustre.

Glucose litmus agar slant. Growth slight, granular, moist and gray. Slight reduction of the litmus within 24 hours and a permanent acidity within 96 hours.

Glucose litmus agar colonies. Colony formation is very slow and the colonies only appear within 48 hours. They are similar to those on agar but less profuse. At times a slight wrinkling occurs.

Gelatin stab Very slow surface liquefaction.

Gelatin colonies. The colonies are definitely circumscribed, gray and moist, under the low power of the microscope showing finely granular centers surrounded by small gray rings. Beyond the rings there are other granular areas the outer borders of which consist of dense gray rings. A very peculiar odor like that of semen plus some aromatic radical occurs in gelatin.

Broth. Peculiar pellicle made up of discrete colonies which sink to the bottom. There is also a slight turbidity.

Peptone. Growth similar to that in broth.

Potato. The growth is dull, moist, gray, granular and finely wrinkled. A slight lustre appears which becomes more pronounced as the medium becomes drier.

Litmus milk. Within 24 hours there is a complete reduction of the litmus with an alkaline ring at the surface. A soft coagulum is formed within 24 hours which is somewhat firmer than the coagulum formed by other spore-bearing organisms. Peptonization begins after 48 hours and requires a considerable period before it is completed. The fluid remaining is straw-colored.

Blood serum. The growth is grayish, moist, non-spreading and not profuse. Within 96 hours it becomes finely wrinkled.

Fermentation tubes. Glucose: there is a heavy pellicle formed and a turbid growth in the bowl and neck of tube. If litmus is present it is reduced in the closed arm. Also a turbid growth is found in this arm. Reaction acid.

Saccharose: a similar condition is apparent with little or no acidity.

Lactose: there is the same reaction with a slight alkalinity.

Thermal death point. The spores survive 12 pounds pressure in the autoclave but are destroyed by 15 pounds pressure. They are destroyed by one hour's steaming in the Arnold sterilizer.

SPORE-BEARING BACTERIA IN SOIL

BY C. A. LAUBACH AND J. L. RICE

In a preliminary investigation of the germ content of soils obtained in the neighborhood of Baltimore the attempt was made to verify the results already obtained by the work on milk, dust and water and to determine whether the types previously isolated from the other sources could also be found in this environment. Altogether some 63 cultures were obtained and found to consist of the following types:

	Baltimore soil
Bacillus cereus Frankland	28
Bacillus mesentericus (Flügge) Migula	15
(*Bacillus mesentericus fuscus* Flügge.)	
Bacillus subtilis (Ehrenberg) Cohn	8
Bacillus vulgatus (Flügge) Trevisan	6
(*Bacillus mesentericus vulgatus* Flügge.)	
Bacillus brevis Migula	3
Bacillus megatherium De Bary	1
Bacillus mycoides Flügge	1
Bacillus petasites Gottheil	1

It is interesting to note the great predominance of *Bacillus cereus* which makes up nearly half the isolations, the relative infrequency of *Bacillus subtilis* and the rarity of *Bacillus megatherium, Bacillus petasites,* and *Bacillus mycoides. Bacillus mesentericus* appears also as a more frequent isolation than *Bacillus vulgatus.*

Eight different samples of raw and heated soil were selected for this examination, some samples being plated immediately, others being incubated at 37°C. for 48 hours before plating. It was hoped that a great diversity of organisms would be obtained by this method and no special attempt was made to estimate with any great accuracy the germ content of any particular sample. In general it was noted however, that heated soil furnished a greater variety of types than unheated soil and that the incubation of the sample was distinctly an unfavorable factor since one type tended to overgrow the other types present. It was thought that the prevalence of *Bacillus cereus* might be due to its power of

crowding out the other microörganisms with which it was associated in soil, rather than to its original presence in greater numbers.

In a subsequent investigation 520 cultures were obtained from 8 soils, 5 of them from the vicinity of Baltimore and 3 from Nazareth, Pennsylvania. The heating of the soil to temperatures of 60° and 80°C., and the incubation of the sample was abandoned owing to the inconsistency of the results obtained and the overgrowth by *Bacillus cereus*. All samples were boiled for 20 minutes and plated immediately. Of the 520 cultures 306 were from Baltimore and 214 from Nazareth. The cultures included the following previously described types:

	Baltimore Soil	Nazereth Soil
B. petasites Gottheil	73	116
B. cereus Frankland	134	41
B. megatherium De Bary	20	13
B. subtilis (Ehrenberg) Cohn	24	9
B. mesentericus (Flügge) Migula (*Bacillus mesentericus fuscus* Flügge.)	9	11
B. vulgatus (Flügge) Trevisan (*Bacillus mesentericus vulgatus* Flügge.)	12	6
B. mycoides Flügge	15	2
B. mesentericus var. *flavus*		9
B. cereus var. *fluorescens*	3	
B. fusiformis Gottheil	3	2
B. brevis		3
B. simplex Gottheil	1	
B. cohaerens Gottheil	1	2
	304	214

One new species (BACILLUS AGRI) belonging to the Cohaerens-simplex group was isolated from the Baltimore soil on two occasions.

In the results obtained from Baltimore it is interesting to note the predominance of *Bacillus cereus*, the large number of isolations of members of the Megatherium-petasites group and the small number of *Bacillus mycoides*. *Bacillus mycoides* indeed is a rare organism in Baltimore and soil conditions here apparently differ markedly from those in other localities. *Bacillus subtilis* and the members of the mesentericus group are also uncommon

while the other organisms mentioned are extremely rare. The conclusions reached by the study of the total number of organisms are borne out by the analysis of the individual samples which may be tabulated as follows:

TYPE	BALTIMORE SERIES					NAZARETH SERIES		
	1	2	3	4	5	6	7	8
B. cereus	16	20	23	17	58	8	10	23
B. petasites	10	18	15	21	9	20	30	66
B. mycoides	5	6	3	1				2
B. megatherium	4	6	8	8	3	3	4	6
B. subtilis	2	4	3	7	8	1	4	4
B. vulgatus	1	2	4	4	1	2	2	2
B. mesentericus			2	6	1		8	3
B. cohaerens				1		1		1
B. fusiformis					3			2
B. agri				1	1			
B. simplex		1						
B. cereus var. *fluorescens*	1		2					
B. mesentericus var. *flavus*							7	2
B. brevis							2	1
Total	39	57	60	66	84	35	67	112

Source of samples: 1, Paving sand, Baltimore City; 2, surface soil, lawn of Johns Hopkins Hospital; 3, soil from fertilized flower bed, Baltimore City, under cultivation, 20 years; 4, soil from cultivated garden, Baltimore; 5, soil from field, suburb of Baltimore; 6, clay from depth of 5 feet, Nazareth; 7, soil from highly fertilized garden, under cultivation more than 25 years; 8, richly fertilized field soil.

In the Nazareth series the predominant organism was *Bacillus petasites* which made up more than half the isolations. *Bacillus cereus* stood next while *Bacillus mycoides* was obtained on but two occasions. The analysis of the individual samples shows the same predominance of *Bacillus petasites* and is chiefly interesting in demonstrating the small number of organisms isolated from soil collected at the depth of five feet as contrasted with the more diversified flora of highly fertilized top garden and field soil.

In general it may be seen that the study of soil confirms the conclusions reached by the study of milk, dust and water. The

predominating types isolated from these latter sources were also found predominating in soil, since the 520 cultures revealed only one new species.

Bacillus agri nov. sp.

This organism was isolated on two occasions from Baltimore soil and cannot be identified with any previously-described species. It evidently belongs in the Simplex-cohaerens group and because of its different cultural reactions must be described as a new species.

Morphology. Large uniform homogeneous rods with round ends measuring 0.625 to 0.75 by 2.5 to 5 microns in young cultures on plain agar. Occasionally chains with a number of elements are found and shadow or transparent forms measuring 12–15 microns in length. On glucose agar there is no special change in morphology, but the proportion of shadow forms is greater. The morphology resembles that of *Bacillus simplex.* (Figures 73 and 74.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form within 24 hours but are not abundant till after the lapse of three or four days. They are central or slightly ex-centric, no wider than the rods from which they spring. The free spores retain rims of protoplasm for some time. They measure 0.625 to 1.125 to 1.25 microns. As they lose their protoplasm they become more oval and measure 0.5 to 0.625 by 0.75 to 1.125 microns. The spore-formation resembles that of *Bacillus simplex.*

Agar slant. Fairly profuse gray moist smooth glistening opaque growth along the line of inoculation with a slight tendency to spread.

Agar stab. Faint granular growth along line of inoculation.

Agar colonies. Small gray moist glistening elevated colonies with opaque centers. No tendency to spread.

Glucose litmus agar. Fairly profuse gray moist smooth glistening opaque growth with a slight tendency to spread. The litmus is completely reduced within 48 hours with a very slight

initial acidity which is rapidly replaced by a permanent alkalinity. The growth wrinkles slightly.

Glucose litmus agar colonies. Grayish, moist, opaque glistening and slightly wrinkled colonies. The reaction is the same as that on the glucose litmus agar slant.

Gelatin stab. A definite granular growth along the line of inoculation followed by complete liquefaction. The liquefaction is funnel-like.

Gelatin colonies. Definitely circumscribed grayish, moist, glistening colonies with opaque centers and thin lace-like peripheries. The colonies rest in excavations caused by liquefaction. On examination with the low power, they appear to be composed of large flocculi.

Broth. A very turbid growth which clears by sedimentation. No pellicle is formed.

Peptone. Growth similar to that in broth.

Potato. Fairly abundant, moist, heaped up, glistening, viscous and grayish growth.

Litmus milk. No change is noticed within 96 hours, after which period a slight reduction becomes evident. The reduction is completed within about 7 days and then the milk is slowly peptonized. Peptonization is generally completed within 14 days. The remaining liquid is at first grayish in color but after a variable period becomes amber-colored.

Blood-serum. A fairly abundant, thin, smooth, slowly spreading, slightly glistening, brownish growth occurs. No liquefaction of the medium.

Fermentation tubes. Glucose: a turbid growth occurs in the bowl and neck but does not extend up the closed arm. No scum is formed and a very slight initial acidity is produced which gives way to a slight alkalinity.

Saccharose: the growth corresponds closely to that in glucose. There is a slight alkalinity.

Lactose: the growth is similar with the production of a slight alkalinity.

Thermal death point. The spores survive 10 pounds in the auto-

clave but are destroyed by 12 pounds pressure. In the Arnold they survive 30 minutes steaming but are destroyed by one hour's exposure.

MISCELLANEOUS CULTURES

BY WILLIAM W. FORD

On a number of occasions miscellaneous cultures from a variety of sources have been studied with care and identified. Such cultures, about 100 in number, include spore-bearing organisms found at times as laboratory contaminations, in the normal and abnormal dejecta of children and adults, in milk products, etc. The majority of such cultures were easily identified and found to correspond to the well-known types already described. The most frequent isolation was *Bacillus cereus* which is our common laboratory contamination. Next to this organism *Bacillus subtilis* was most frequent, then *Bacillus vulgatus* or *Bacillus mesentericus*. Members of the Megatherium-petasites group were also not uncommon. In addition to such isolations as these *Bacillus circulans* of Jordan was found on one occasion. It is recognized by its cylindrical terminal spores and by its cultural reactions. Dr. Jordan was kind enough to examine the culture and confirmed our identification. *Bacillus circulans* is evidently one of the rarer sporulating bacteria. At another time a round-spored organism resembling *Bacillus tetani* in morphology was encountered as a contamination on an old agar plate. This species was apparently first described by Kruse (Flügge, 1896) as an aerobic variety of the tetanus bacillus. On three occasions an organism was isolated which was originally obtained by Flügge (1894) and named later *Bacillus brevis* by Migula (1900). The same species was found by us in Montreal. Finally on one instance a culture was found in the normal dejecta which could not be identified with any previously described organisms. The morphology and spore formation were so characteristic and so different from that seen with other spore-bearing bacteria that it was decided to create a new species for which the name BACILLUS CENTROSPORUS is selected. A detailed description of these organisms follows.

Bacillus circulans Jordan 1890

This organism was described originally by Jordan in 1890. The present culture was obtained from normal human dejecta and corresponds closely with Jordan's description.

Morphology. Rather long and thin bacilli with rounded or square ends measuring about 0.5 by 2.5 to 4 microns in 24 hour agar cultures. Occurs usually as single cells. Protoplasm homogeneous. On glucose agar the organisms are thicker and longer measuring 0.75 by 5 to 8 microns. (Figure 75.)

Motility, Active progressive and rotatory motility in 24 hour agar cultures.

Staining properties. Gram-positive.

Spore formation. Spores form early on plain agar often in 24 hours. Abundant in 48 hours. Form more slowly on glucose agar. They are cylindrical, wider than the rods from which they spring and terminal in position. The free spores may retain rims of protoplasm at one end or may be naked. They measure usually about 0.75 by 1.125 microns.

Agar slant. Thin slightly moist non-spreading growth, pale white in color.

Agar colonies. Round regular non-spreading colonies, under low power pale yellow, finely granular, with entire edges. Older colonies become irregular and amoeboid showing under low power central nuclei and thin peripheries.

Agar stab. Faint growth along line of inoculation spreading at point of puncture.

Glucose agar. Faint thin non-spreading growth producing an acid reaction beginning in 24 hours and quite marked at the end of 5 to 6 days.

Glucose agar colonies. Small fine colonies, under low power pale yellow, granular with entire or serrated edges. Reaction acid.

Gelatin stab. Faint growth along line of inoculation. Little or no surface growth. No liquefaction.

Gelatin colonies. Small fine colonies with hazy outlines, under low power dark opaque with fuzzy margins. Older colonies quite amoeboid. No liquefaction.

Broth. Slight turbidity, no scum, no sediment.

Peptone. Slight turbidity, no scum, no sediment.

Potato. No visible growth.

Milk. Complete decolorization within 24 to 48 hours. Gradual production of acidity and late coagulation, which may appear spontaneously and always occurs if the milk be heated.

Blood serum. Faint thin white non-spreading growth. No liquefaction.

Fermentation tubes. Glucose: turbidity in bowl and closed arm. No scum. Reaction acid, appearing in 3 to 4 days.

Saccharose: appearance the same. Reaction acid.

Lactose: appearance the same. Reaction acid.

Thermal death point. Spores destroyed by steaming 15 minutes in the Arnold. Survive 18 pounds pressure in the autoclave. Destroyed by 20 pounds pressure.

Bacillus pseudotetanicus (Kruse) Migula 1900

This organism was first described by Kruse in the 3rd edition of Flügge's "Die Mikroorganismen" under the name *Bacillus pseudotetanicus* var. *aerobius.* It resembled *Bacillus tetani* in morphology and spore-formation but was distinguished from it by its aerobic development, its failure to liquefy and its lack of pathogenicity. Apparently the same organism has been described by Neide (1904) as *Bacillus sphaericus.* The species here described has the morphological and cultural features given by both Kruse and Neide and under the rules of nomenclature the name given by Migula should be adopted. It was found but once as a contamination on an agar plate.

Morphology. Short thick bacilli with rounded ends occurring as single cells or as two cells end to end. In 24 agar cultures they measure 0.75 by 1.5 to 3 microns. Protoplasm homogeneous. Many of the cells are fusiform, distinctly swollen toward the middle while others are swollen at the ends. On glucose agar the organisms are longer and thicker measuring 1.125 by 1.5 to 4 microns. They show no change in the character of the protoplasm. In older cultures the organisms often form long threads measuring 8 to 15 microns in length. (Figures 76 and 77.)

Motility. Active and progressive rotatory motility in 24 hour agar cultures.

Staining properties. Gram-negative.

Spore formation. Spores are formed early often within 24 hours on both plain and glucose agar. They are round, and usually appear at the ends of the organisms in a sub-terminal position, but may be distinctly terminal. The spores are of greater diameter than the vegetative rods from which they spring and thus give a clavate or club-shaped appearance to the organisms. The organisms taken from glucose agar resemble markedly the tetanus bacillus cultivated on this medium. The free spores retain fairly thick walls of protoplasm for some time. They vary in size from 1 to 1.125 microns in diameter.

Agar slant. Moist rather thin translucent growth in 24 hours, becoming pale yellowish white in old cultures. No tendency to spread except when the medium is very moist.

Agar stab. Faint growth along line of inoculation with slight spreading on the surface. In older cultures the surface growth is thicker and more abundant but does not usually spread far from the line of puncture. Easily scraped from medium.

Agar colonies. Colonies round and regular or amoeboid in 24 hours. Under low power they show dark central areas with lighter peripheries. As they age they become thicker and denser with more pronounced central nuclei.

Glucose agar. Pale white moist rather thin growth in 24 hours, becoming pale yellow or cream yellow in older cultures.

Glucose agar colonies. In 24 hour plates the colonies are irregular, amoeboid, thick, showing under low power darker central nuclei with lighter peripheries but with entire edges. As the colonies become older they become denser and thicker with heaped-up edges. They give a peculiar bizarre appearance to the plate as though it was covered with drops of moisture. Reaction of agar remains neutral or becomes alkaline.

Gelatin stab. Faint line growth appearing after 48 to 72 hours with a slightly spreading growth on the surface. In older cultures the surface growth spreads considerably from the point of puncture on top of the gelatin which remains solid. Rarely the line growth becomes arborescent.

Gelatin colonies. Small fine colonies visible only after 48 hours. Under low power they are round, regular and pale yellow with entire margins. As they grow older they become denser and more opaque and under low power show central nuclei with pale margins. Colonies two weeks old are thick brownish non-spreading. No liquefaction.

Broth. Turbidity and rather faint sediment. No scum.

Peptone. Turbidity with slight sediment. No scum.

Potato. Rather scanty yellowish moist growth becoming brown in old cultures.

Litmus milk. No change in appearance of the milk in the first few days then a gradual reduction of the litmus, completed in 15 to 18 days. No peptonization even in milk cultures kept under observation four weeks. No coagulation.

Blood serum. Dry thin yellowish growth becoming pale yellow-brown in old cultures. No liquefaction.

Fermentation tubes. Glucose: turbidity in bowl, no scum, arm clear. Reaction alkaline.

Saccharose: reaction the same.

Lactose: reaction the same.

Thermal death point. The spores resist steaming in the Arnold sterilizer 15 minutes but are destroyed in 30 minutes. They resist a pressure of 14 pounds in the autoclave but are destroyed by 16 pounds.

Bacillus brevis Migula 1900

This organism was first described by Flügge (1894) as BACILLUS No. I. According to Neide (1904) who has given an accurate account of its morphology and cultural reactions under the name *Bacillus lactis* Flügge, it is probably identical with *Bacillus cylindrosporus* Burchard (1898). The correct name is that of Migula. Three different strains have been isolated from milk, from soil, and from dust. They agree in their cultural reactions with the description given by one of us (W. W. F.) of a culture isolated in Montreal.

Morphology. Bacilli with pointed ends and slightly granular protoplasm, sometimes fusiform in shape. In 24 hour cultures on plain

agar they measure 0.375 to 0.5 by 2 to 3 microns. On glucose agar they are more uniform and slightly thicker and longer, measuring usually 0.5 by 3 to 4 microns. (Figures 78, 79 and 80.)

Motility. Active progressive motility in 24 hour agar cultures.

Staining properties. Gram-negative.

Spore formation. Spores are formed early appearing often within 24 hours on plain and glucose agar. They are cylindrical, usually subterminal in position, thicker than the organisms from which they spring, and present a characteristic appearance. They may retain spurs of protoplasm at each end or may be entirely naked. They measure usually 0.75 by 1.125 to 1.5 microns. Rarely they may be distinctly terminal in position.

Agar slant. Thick moist spreading growth with a silvery sheen abundant in 24 hours and becoming thick white and glistening in older cultures. Easily scraped from medium.

Agar stab. Filiform growth along line of inoculation with an abundant surface growth spreading to the tube wall.

Agar colonies. Surface colonies in 24 hours are round and regular or thin spreading translucent, under low power slightly granular with entire edges. Deep colonies small fine, under low power irregular and pale yellow. As the superficial colonies get older they become thicker and heaped up slightly. The deep colonies may show irregular fuzzy margins under the low power.

Glucose agar. Heaped up white moist non-spreading growth in 24 hours, becoming thick and raised in old cultures. Reaction alkaline.

Glucose agar colonies. Superficial colonies are slightly thicker than plain agar colonies in 24 hours and show a greater tendency to spread. Under the low power they are granular with entire edges. Deep colonies small and fine, under low power irregular and pale yellow. Older superficial colonies are thick, irregular and heaped up. Reaction of agar alkaline.

Gelatin stab. Faint growth along line of inoculation and at the point of puncture in 3 to 4 days, with beginning liquefaction. Distinct cup-shaped or funnel liquefaction in two weeks. Occasionally a fragile scum appears on the liquid gelatin.

Gelatin colonies. Colonies begin to appear in 48 hours and show

dark central areas with hazy peripheries. Under the low power they show dark opaque centers with wavy branching peripheries. Deep colonies frequently show peculiar prickly margins. Gelatin completely liquefied at the end of about two weeks and may show a thick scum on the surface.

Broth. Turbidity and a flocculent sediment in 24 to 48 hours with a gradual production of a heavy scum which appears often only after 4 days. In old cultures the precipitate is quite heavy.

Peptone. Turbidity and heavy flocculent sediment. Scum may be thick and heavy or entirely lacking.

Potato. Usually a rather scanty moist yellow or yellow-brown growth.

Milk. No change in reaction. Litmus begins to decolorize in 48 hours and by the end of two weeks is completely decolorized. At the same time the proteins are digested and the resulting fluid is a pale rather thin colorless solution. No coagulation.

Blood serum. Thin cream-white non-spreading growth becoming yellowish-brown in old cultures. No liquefaction.

Fermentation tubes. Glucose: thick scum, turbidity in bowl, arm clear. Reaction alkaline.

Saccharose: reactions the same.

Lactose: reactions the same.

Thermal death point. The spores survive steaming 30 minutes but are destroyed by 45 minutes exposure. They may survive a pressure of 16 pounds in the autoclave but are destroyed by 18 pounds pressure.

Bacillus centrosporus nov. sp.

This is a new species obtained but once, from the normal dejecta of a child from the Harriet Lane Home.

Morphology. In 24 hour cultures on plain agar at 37°C. or in 36 hour cultures at 22°C. long thin bacilli with pointed ends fusiform or clostridium in shape with slightly granular protoplasm (grassy), occurring usually as single cells measuring 0.375 to 0.5 by 3 to 4.5 microns in dimensions. The majority of the

organisms measure about 3 to 4 microns in length. On glucose agar the organisms measure about 0.375 by 2.25 to 3 microns and the ends are rounder but there is no change in the character of the cytoplasm. (Figures 81 and 82.)

Motility. Active progressive and rotatory motility in 24 hour agar culture.

Staining properties. Organisms stain readily by the ordinary dyes. Gram-negative.

Spore formation. Spores are formed early appearing in 24 hours on plain agar in the thermostat, somewhat later on glucose agar. They are usually formed in the central portion of the rods causing a distinct swelling so that the bacilli become spindle-shaped. Rarely the spores appear toward the end of the rods in a subterminal position or they may even be terminal. They are much wider than the vegetative rods and when free are distinctly cylindrical with pointed spurs. They measure 0.625 by 1.125 to 1.5 microns. By the end of 48 hours both at 37° and at 22° the spores are abundant.

Agar slant. Thick moist rather dark non-spreading growth in 24 hours, becoming thicker and raised in 48 hours and in older cultures. Easily scraped off from medium.

Agar stab. Growth along line of inoculation in 24 hours, gradually spreading on the surface of the agar at the point of puncture.

Agar colonies. Round regular rather translucent colonies in 24 hours, granular with smooth margins under the low power. Older colonies show pale central areas with heaped up peripheries but remain small regular and slightly raised, resembling those of *Bacillus coli.*

Glucose agar slant. Thick moist cream-yellow non-spreading growth in 24 hours, becoming thick heavy with an irregular surface of a reddish brown color in old cultures. Reaction alkaline.

Glucose agar colonies. Irregular heaped up colonies in 24 hours tending to become slimy or viscid-looking. Under low power they are irregular, heaped up or swollen, with entire margins. At times they have a dew-drop appearance. On old cul-

tures (10 days) they are thicker and slightly brownish. Reaction of medium alkaline.

Gelatin stab. Filiform growth along line of inoculation and gradual liquefaction in the form of a sphere below the surface. Liquefaction is slow, taking place only after 7 to 8 days. Liquefaction gradually reaches the walls of the tube. Growth not arborescent.

Gelatin colonies. Colonies appear only after 5 to 6 days. They are small fine and surrounded by a hazy zone. Gelatin slowly liquefied. Under low power colonies are round regular finely granular with entire margins or may show irregular outlying strands.

Broth. Turbidity within 24 to 48 hours with a faint friable scum appearing in about 10 days.

Peptone. Turbidity within 24 to 48 hours with faint friable scum in 10 days.

Potato. Moist yellow growth within 24 hours gradually becoming heaped up and irregular and assuming a brown or reddish brown color.

Litmus milk. No change in reaction except for an occasional partial reduction of the litmus. No peptonization. No coagulation.

Blood serum. Pale yellow growth in 24 hours gradually becoming thick and moist in old cultures. No liquefaction.

Fermentation tubes. Glucose: turbidity in bowl, no scum, arm clear. Reaction alkaline.

Saccharose: reactions the same.

Lactose: reactions the same.

Thermal death point. The spores stand boiling 15 minutes but are destroyed by boiling for 30 minutes. They resist 15 minutes' exposure in the Arnold but are destroyed by 30 minutes' exposure. They resist autoclaving at a pressure of 12½ pounds but are destroyed by 14 pounds pressure.

CLASSIFICATION

BY W. W. FORD

From a study of spore-bearing organisms lasting over a period of four years, during which over 1700 cultures were worked out from a variety of sources including milk, dust, water, soil, intestinal contents, and laboratory stock cultures 28 species or varieties of species were isolated and established as distinct types by the usual morphological, developmental, tinctorial and cultural features available for systematic investigation. The majority of these types were encountered on many separate occasions and our descriptions of them are based upon those fixed and permanent characters which we regard as establishing bacterial species. These types thus represent the organisms met with in our work quite regardless of their identification as previously established species or varieties. Of the total number, 28, we were able to identify 22 as species already described in the literature. Whether these species are correctly named, is a matter about which of course opinions may vary. We feel however that our identification of these 22 species is as satisfactory as the confusion in the literature and the uncertainty of stock cultures permit. In addition to the 22 types described by previous workers two distinct varieties of old species were found while four organisms were isolated which are so different from any described in the literature as to merit establishment as new species. To these six organisms new names have been given. With these 28 types in mind it now becomes possible to classify the spore-bearing organisms from a preliminary standpoint. The basis for the classification rests primarily upon morphology, spore-formation and motility, and secondarily upon tinctorial and cultural features. All the characters are easily demonstrated in any properly equipped bacteriological laboratory. Both the microchemical properties of the bacterial cells and the methods of spore-germination while studied when possible have been disregarded in arranging the species in groups. The extent to which this classification is of permanent value will be shown, we believe, by the approval or disapproval with which it is received by American bacteriologists.

AEROBIC SPORE-BEARING NON-PATHOGENIC BACTERIA

Group I. Subtilis group

Small homogeneous sluggishly motile organisms measuring 0.375 by 1.5 to 2.5 microns. No threads on glucose agar. Central or ex-centric spores, oval, measuring 0.5 by 0.75 to 0.875 microns, often retaining terminal tags of protoplasm. Growth on solid media hard and penetrating, with tenacious scums on fluid media.

Represented by

Bacillus subtilis Cohn.

Bacillus subtilis-viscosus Chester.
(Characterized by viscosity.)

Group II. Mesentericus group

Small homogeneous actively-motile organisms measuring 0.5 by 2 to 4 microns. Often produce long threads on glucose agar. Spores measure 0.5 by 1 to 1.125 microns, oval, retaining terminal tags of protoplasm. Growth on hard media as soft pultaceous mass with tendency to wrinkle, on fluid media as friable easily-broken scums.

Represented by

Bacillus vulgatus (Flügge) Trevisan.
(*Bacillus mesentericus vulgatus* Flügge.)

Bacillus mesentericus (Flügge) Migula.
(*Bacillus mesentericus fuscus* Flügge.)

Bacillus aterrimus Lehmann & Neumann.
(*Bacillus mesentericus niger* Lunt.)

Bacillus globigii Migula.
(*Bacillus mesentericus ruber* Globig.)

Bacillus niger Migula.
(*Bacillus lactis niger* Gorini.)

Bacillus mesentericus var. *flavus*.

Bacillus panis Migula.
(*Bacillus mesentericus panis viscosi I* Vogel.)
(Motility lost by capsule formation.)

Group III. Cohaerens-simplex group

Motile organisms somewhat larger than either *Bacillus subtilis* or *Bacillus mesentericus*, measuring 0.375 to 0.75 by 0.75 to 3 microns. Thicker and longer forms on glucose agar. Involution and shadow forms common and appear early. Spores cylindrical, measuring 0.5625 to 0.75 by 1 to 1.5 microns. Growth as a soft mass on hard media, as turbidity with little or no scum on fluids.

Represented by

Bacillus cohaerens Gottheil.
Bacillus simplex Gottheil.
BACILLUS AGRI NOV. SP.

Group IV. Mycoides group

Large organisms with square ends growing in long chains. Single cells measure 0.5 by 3 to 6 microns. On glucose agar organisms are thicker and longer and made up of globular bodies. Tendency for organisms to grow in curves or spirals. Spores central or ex-centric, round or oval to cylindrical, measuring 0.75 to 1 by 1 to 2 microns. Spores vary greatly in size and often appear in chains. Growth on hard media dry and penetrating, on fluid media as firm tenacious scum.

Represented by

Bacillus mycoides Flügge.
Bacillus prausnitzii Trevisan.
(*Bacillus ramosus liquefaciens* Prausnitz.)
BACILLUS ADHAERENS NOV. SP.
(No motility.)

Group V. Cereus group

Large motile organisms with round ends measuring 0.75 by 2.25 to 4 microns. Tend to grow in short chains. Thicker and longer on glucose agar where protoplasm is converted into globular bodies. Central or excentric spores, cylindrical, measuring 0.5 to 0.75 by 1.125 to 1.5 microns. Spores retain proto-

plasm at one or both ends often resembling enlarged subtilis or mesentericus spores. Growth on hard media as soft pultaceous mass with tendency to fold or wrinkle, on fluid media as thick friable scum.

Represented by

Bacillus cereus Frankland.
Bacillus albolactus Migula.
Bacillus cereus var. *fluorescens* nov. var.

Group VI. Megatherium group

Very large actively motile organisms measuring 0.75 to 1.25 by 3 to 9 microns. Often in long forms which spread out, lose their cytoplasm and show peculiar aggregations of protoplasm at the periphery. Protoplasm rapidly converted into peculiar globular highly refractile bodies, particularly on glucose agar. Shadow and transparent forms appear early. Spores central, excentric or sub-terminal, oval to cylindrical, measuring usually 0.75 to 1.125 by 1.5 to 2 microns. Spores vary greatly in shape, sometimes round, sometimes rectangular, often reniform. Growth on solid media as thick pultaceous mass, on fluid media as turbidity with little or no scum formation.

Represented by

Bacillus megatherium De Bary.
Bacillus petasites Gottheil.
Bacillus ruminatus Gottheil.

Group VII. Round terminal spored group

Small actively motile organisms measuring 0.5 to 0.75 by 1.5 to 3 microns, often forming long threads in old cultures. Protoplasm homogeneous. Spores subterminal or terminal, round, thicker than the organisms from which they spring, measuring 1 to 1.5 microns in diameter.

Represented by

Bacillus pseudotetanicus (Kruse) Migula.
(*Bacillus pseudotetanicus* var, *aerobius* Kruse.)
Bacillus fusiformis Gottheil.

Group VIII. Cylindrical terminal spored group

Small thin actively motile organisms measuring 0.375 to 0.5 by 2.5 to 4 microns. Slightly larger on glucose agar but no change in character of protoplasm. Spores terminal, cylindrical, measuring usually 0.75 by 1.125 to 1.5 microns.

Represented by

Bacillus circulans Jordan.
Bacillus brevis Migula.
Bacillus terminalis Migula.

Group IX. Central spored group

Long, actively motile organisms with pointed ends measuring 0.375 to 0.5 by 1.125 to 4 microns. Slightly larger on glucose agar, but no change in character of protoplasm. Spores develop in the middle of the rods which become spindle-shaped. Spores large cylindrical measuring 0.625 to 0.875 by 1.125 to 1.5 microns.

BACILLUS CENTROSPORUS nov. sp.
BACILLUS LATEROSPORUS nov. sp.

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LIST OF ILLUSTRATIONS

PLATE 1

FIG. 53. *Bacillus prausnitzii* from dust. (*Bacillus ramosus liquefaciens.*) Plain agar, 6 hours

FIG. 54. *Bacillus prausnitzii* from dust. (*Bacillus ramosus liquefaciens.*) Plain agar, 48 hours

PLATE 2

FIG. 55. *Bacillus mesentericus* var. *flavus* from dust. Plain agar, 6 hours.

FIG. 56. *Bacillus mesentericus* var. *flavus* from dust. Glucose agar, 24 hours

PLATE 3

FIG. 57. *Bacillus mesentericus* var. *flavus* from dust. Plain agar, 12 days

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PLATE 4

FIG. 59. *Bacillus ruminatus* from dust. Plain agar, 16 days

FIG. 60. *Bacillus panis* from dust. (*Bacillus mesentericus panis viscosi I.*) Plain agar, 6 hours

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PLATE 6

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PLATE 7

FIG. 65. *Bacillus subtilis* var. *viscosus* from water. Plain agar, 6 hours

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PLATE 8

FIG. 67. *Bacillus subtilis* var, *viscosus* from water, Plain agar, 13 days

FIG. 68. *Bacillus cereus* var, *fluorescens* from water. Plain agar, 6 hours

PLATE 9

FIG. 69. *Bacillus cereus* var. *fluorescens* from water. Plain agar, 24 hours

FIG. 70. *Bacillus cereus* var. *fluorescens* from water. Glucose agar, 24 hours

FIG. 53

FIG. 54

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 55

FIG. 56

(Laubach, Rice and Ford; Aerobic Spore-bearing Non-pathogenic Bacteria)

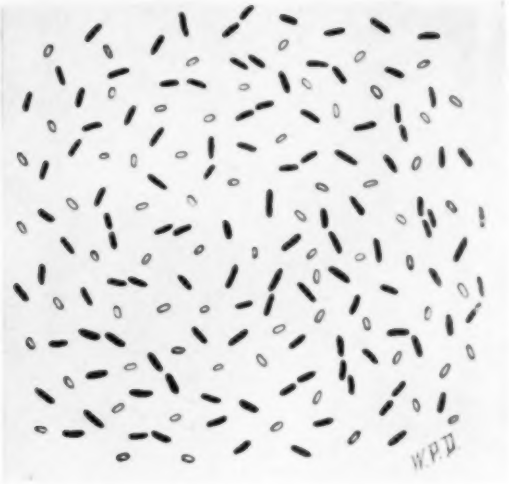

FIG 57

FIG. 58

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 59

FIG. 60

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 61

FIG. 62

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 63

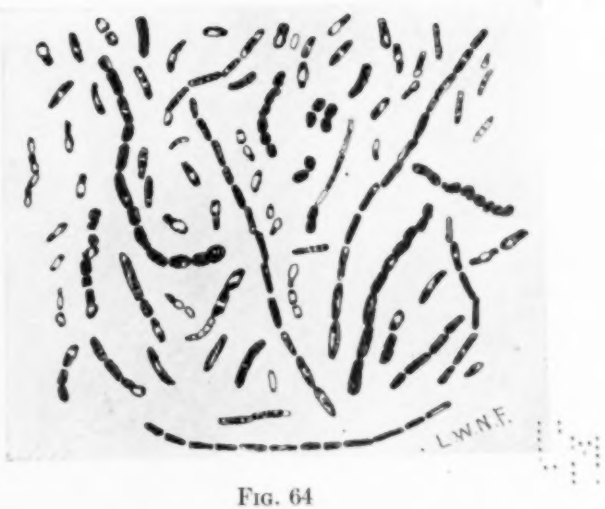

FIG. 64

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 65

FIG. 66

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 67

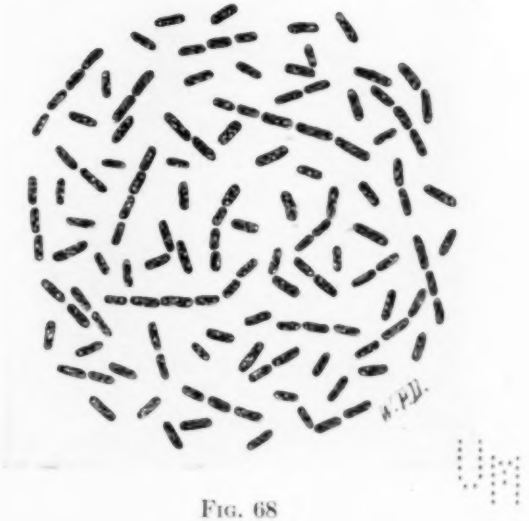

FIG. 68

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 69

FIG. 70

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

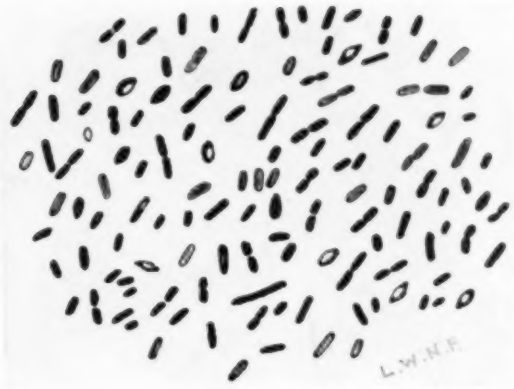

FIG. 71

FIG. 72

FIG. 73

FIG. 74

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 75

FIG. 76

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 77

FIG. 78

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

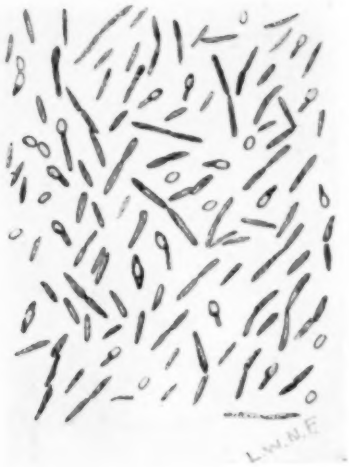

FIG. 79

FIG. 80

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

FIG. 81

FIG. 82

(Laubach, Rice and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

PLATE 10

FIG. 71. BACILLUS LATEROSPORUS from water. Plain agar, 6 hours
FIG. 72. BACILLUS LATEROSPORUS from water. Plain agar, 3 days

PLATE 11

FIG. 73. BACILLUS AGRI from soil. Plain agar, 24 hours.
FIG. 74. BACILLUS AGRI from soil. Plain agar, 3 days.

PLATE 12

FIG. 75. *Bacillus circulans* from human dejecta. Plain agar, 24 hours.
FIG. 76. *Bacillus pseudotetanicus* from contaminated plate. Plain agar, 20 hours.

PLATE 13

FIG. 77. *Bacillus pseudotetanicus* from contaminated plate. Glucose agar, 18 hours.
FIG. 78. *Bacillus brevis* from dust. Plain agar, 24 hours.

PLATE 14

FIG. 79. *Bacillus brevis* from soil. Plain agar, 48 hours.
FIG. 80. *Bacillus brevis* from milk. Plain agar, 3 days.

PLATE 15

FIG. 81. BACILLUS CENTROSPORUS from human dejecta. Plain agar plates, 30 hours.
FIG. 82. BACILLUS CENTROSPORUS from human dejecta. Plain agar, 24 hours.

A RAPID AND SIMPLE INDOL TEST

PAUL R. CANNON

From the Department of Hygiene and Bacteriology, The University of Chicago

It is well known that tryptophane in an inorganic solution furnishes an excellent medium for the demonstration of indol production by bacteria. Zipfel (1) in 1912 found that indol could be demonstrated by this method at the end of twenty-four hours, giving the best reaction with p-dimethyl-amido-benzaldehyde. The test is made by adding to the culture to be tested one cubic centimeter of a solution consisting of p-dimethyl-amido-benzaldehyde, 4 parts; absolute alcohol, 380 parts; and concentrated hydrochloric acid, 80 parts.

The expense of preparing tryptophane and the great difficulty in obtaining it at all in the United States at the present time are drawbacks to the introduction of the tryptophane method. As a substitute, I have found that hydrolyzed casein can be used. Cow casein contains about 1.5 per cent tryptophane, which may be obtained in its amino-acid form by hydrolyzing the casein.

The method is as follows: 10 grams of casein are hydrolyzed by 200 cc. of 10 per cent sulphuric acid, the mixture being kept on the water bath for twenty-four hours. At the end of this time, the casein is completely dissolved and the solution is a dark brown. Next, the solution is neutralized by the addition of saturated barium hydrate, thus precipitating out the sulphate. The resulting solution is then evaporated until the amino-acids crystallize. Half of the crystalline mass is dissolved in 500 cc. of Zipfel's inorganic solution consisting of Asparagin and Ammonium lactate, 5 grams each; Potassium acid phosphate, 2 grams; Magnesium sulphate, 0.2 grams; and distilled water, 1000 grams. The medium is tubed and sterilized. Assuming that there are 0.15 grams of tryptophane in the cow casein, tryptophane should be present in the mixture to the extent of about 0.03 per cent.

Tubes of media prepared in this way were inoculated with known indol-forming bacteria, and, at the end of eighteen hours, the indol test was made by using p-dimethyl-amido-benzaldehyde. A pronounced red color almost instantly appeared, showing the presence of indol. The control and non-indol-formers remained a straw color after the addition of the aldehyde. It proved unnecessary in my tests to use amyl alcohol to dissolve out the color, although this may be done in case indol formation is doubtful.

The brief time—eighteen to twenty-four hours—necessary for the test by the above method, is a great improvement over the old standard peptone test, which required five days. Furthermore, hydrolyzed casein can easily be obtained or prepared, and the constituents of the inorganic solution are available in most laboratories.

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BACTERIAL NUTRITION: A BRIEF NOTE ON THE PRODUCTION OF EREPSIN (PEPTOLYTIC ENZYME) BY BACTERIA

NATHAN BERMAN AND LEO F. RETTGER

From the Sheffield Laboratory of Bacteriology and Hygiene, Yale University

In a recent publication (1) the authors stated that gelatin-non-liquefying bacteria of the *Bacillus coli* type are able to exert but little, if any, proteolytic action on Witte's peptone and on partially purified proteoses. The biuret test was employed in the earlier experiments, and the results were not expressed in mathematical terms. In later studies the changes in the protein content of the media have been recorded in definite figures. Furthermore, the Sörensen method for the determination of amino acids has been used along with the biuret test.

The published results in a few instances gave unmistakable evidence of a reduction in the amount of biuret-giving substances in the media containing Witte's peptone, when *B. coli*, *B. typhi* and *B. pullorum* were employed. These reductions were only slight, however, and were noticeable only after two weeks' incubation. While the changes in the protein content of the media suggested the possibility of ereptic enzyme action, no definite conclusion was warranted. Subsequent investigation has, in a measure at least, clarified the situation.

In a medium containing 1 per cent peptone, 0.25 per cent Liebig's meat extract and 0.5 per cent of sodium chloride *B. coli*, *B. typhi*, and *B. paratyphi A*, under optimum cultural conditions have slowly acted upon the biuret-positive substances in the peptone. While at least two weeks were usually required to produce any appreciable decrease in the "protein," the loss amounted to from 10 per cent to 40 per cent of the original when the digestion was allowed to continue for at least three weeks.

It was impossible to bring about a decomposition of more than

40 per cent of the original "protein" in the peptone solution, even under the most favorable conditions, as for example the addition of ammonium sulphate, asparagine, glycerine, phosphates, etc. In some instances 10 to 20 per cent marked the maximum reduction. When other peptones were employed, however, namely five different American brands, reductions considerably in excess of 40 per cent of the protein were frequently obtained. In all of these experiments the Sörensen method gave corroborative results.

Media containing Witte's peptone which had been dialyzed for at least two days underwent less change in their protein content than the corresponding solutions of untreated peptone. Furthermore, the so-called "proteose fraction" obtained from Witte's peptone by precipitation with ammonium sulphate, repeated precipitation with sodium sulphate, and prolonged dialysis, was apparently unattacked when used in place of the original commercial peptone. The results obtained thus far indicate that the gelatin-non-liquefying organisms of the Coli-typhi-paratyphi group are unable to utilize or in anyway affect the proteose portion of commercial peptones.

In the light of the researches of Emil Fischer and his pupils (2) on polypeptides, the deportment of the *Bacilus coli* type of gelatin-non-liquefying bacteria towards commercial peptone may be explained as follows. These peptones, far from being relatively simple, are mixtures of many organic substances of varying composition and complexity, particularly amino acids and polypeptides. Some of these polypeptides are in all probability quite simple, as for example the condensation product of two simple amino acids, and if we accept the conclusions drawn by different investigators, others are of a much more complex type, and are tied up with the peptone and proteose molecules. Some of the polypeptides give a positive biuret reaction, even the relatively simple ones, while others are negative to this test. All may on decomposition yield ammonia, amines and amino acids.

The proteose fraction of commercial peptone, it may be assumed, contains the relatively complex polypeptides, and hence

is less readily attacked and utilized as food by bacteria than the remainder of the mixture. On the other hand, the real peptone fraction is undoubtedly a more heterogeneous mixture of polypeptides, some of which are indeed quite simple, and are easily broken up by bacteria or their enzymes. In the proteolysis which takes place slowly under most favorable conditions, the simpler polypeptides are attacked. When these have been exhausted so-called "peptolysis" ceases.

The above explanation appears all the more probable from the fact that as bacterial decomposition continues there is a change in the biuret-giving ingredients of the peptone which is indicated by a difference in the color obtained. While the original peptone medium gives a pink color, the reaction changes if there is appreciable peptolysis, and the color becomes distinctly violet and indistinguishable from the biuret color obtained with proteoses and some of the higher proteins.

The peptolysis which is brought about by *Bacillus coli* and its close allies is undoubtedly the result of an ereptic enzyme (erepsin). This enzyme differs, however, from erepsin of animal origin in that it does not attack casein. In comparison with intestinal erepsin, and with the proteolytic enzymes of *B. subtilis*, *B. prodigiosus* and other strong gelatin-liquefying bacteria, in so far as peptolytic action is concerned, the erepsin elaborated by the Coli-typhi-paratyphi group of organisms is decidedly weak.

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A PRACTICAL METHOD FOR THE IDENTIFICATION OF GUINEA-PIGS UNDER TREATMENT

A. PARKER HITCHENS

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The problem of identifying guinea-pigs under treatment is a simple one if only a few animals are in use; the popular method of recording their colors may be adequate; or, separate cages or pens may be provided for each animal. But in laboratories where ten or more animals are inoculated every day a more highly systematized method is required.

Celluloid or aluminum ear tags are both convenient and satisfactory for a small number of animals; they are not easily lost unless two or more full grown male pigs are kept in the same pen. The ear tag method, however, has the disadvantage of consuming considerable time. Furthermore it involves an extra item of stock to look after; if the tags happen to be all used, there may be some difficulty and annoyance in finding a substitute.

Another method in common use is a pictorial description of the animal. On the record sheet is printed the outline of a guinea-pig with the regions of the body indicated. The colors are then noted on the diagram according to their regional distribution. In some laboratories, a rubber stamp is used as the basis for the pictorial description, an arrangement which may be desirable when the same record sheet is to be used for other animals. Many laboratory workers merely draw an elongated oval with little marks at one end to represent eyes and ears and record on this simple diagram the color distribution. All these pictorial methods are reliable, but so much space is required for the *esquisse* that the record of a large amount of work becomes extremely bulky.

The most valuable method for practical purposes would seem to be one whereby (1) ears tags and other equipment are rendered unnecessary, (2) the description is recorded on a single line, on ordinary paper without special ruling or diagram. Although systems similar to the one about to be described have probably been in use for some time, none has appeared to the writer so satisfactory as the elaboration of what was originally merely a makeshift series of abbreviations invented at the time for a particular purpose. The fundamental idea of the plan in question was conceived by Dr. Joseph McFarland more than fifteen years ago; and with a few modifications by the writer, the same method has been used in these laboratories ever since that time. The readiness with which an assistant of no training can learn to describe and recognize guinea-pigs accurately seems to be sufficient recommendation for a more widespread use of this system of abbreviations.

The rules according to which guinea-pigs are described and identified in the Glenolden laboratories are as follows:

IDENTIFICATION OF GUINEA-PIGS

1. Not more than 5 to 8 guinea-pigs are kept in one pen.
2. The assistant in describing the animals mentions first the weight, and then the sex, before calling out the description.
3. The sex is denoted thus:

Male	♂
Female	♀

4. The sides (Right and Left) and the colors are represented in abbreviations by the capitalized initial letter.

The sides of the animal are written thus:

Right	R[1]
Left	L.

The colors are recorded as follows:

[1] The difference between the abbreviations for red, "R" and right, "R", are to be noted.

White	W
Cream	C
Yellow	Y
Silver (silver agouti)	S[2]
Silver and yellow	SY
Red	R
Red and Gold	RG
Gold (golden agouti)	G
Fawn (also chocolate)	F
Yellow and Slate	YS
Slate	S
Red and Black	RB
Black	B

5. The varieties of coat are described thus:

Hair Clipped	C
Rough	Ro
Angora	G

6. The regions of the body are represented by small letters as follows (see fig. 1):

	Singular	*Plural*
Nose	n	
Face	e	
Head	d	
Eye	o	ii
Ear	r	rr
Root (of ear)	√	√
Shoulder	s	ss
Pleura	p	pp
Hip	h	hh
Flank (pleura and hip)	f	ff
Sacrum	x	

7. The pigs are described in the order of the depth of their coloring, a white pig, for instance, is mentioned first, then a pig with few markings over only a small part of the body. Pigs of solid colors come last in the order given above. White is mentioned only when the entire pig is white.

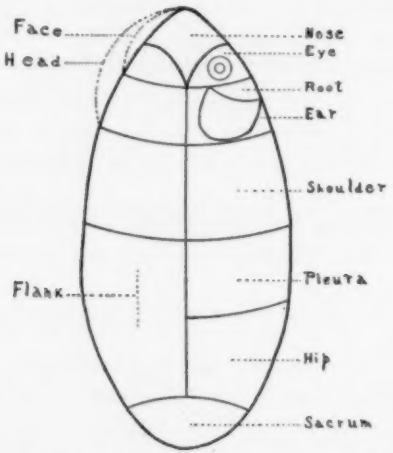
8. In describing an animal, the assistant starts with the fore part of the body on the right side (R); he mentions the color—say, of the right eye—and proceeding backward enumerates the

[2] Note the difference between ·S· (silver) and ·S· (slate).

parts having this color, first on the right side then on the left (provided, of course, this color is distributed on both sides); he then mentions the next color in the same order from the head backward.

9. In the case of two pigs with mixed colors over the entire body it is convenient to note the two colors and follow this by a characteristic difference. Occasionally there may be several pigs

Figure I.



exactly or almost exactly alike—white, for instance, or red and black. In such cases, it may be necessary to clip the hair on the nose or some other part of the body.

10. The description of 8 guinea-pigs in a certain pen might be written as follows:

W	.....White
RRi	.....White with red about the right eye
SlRiLyRp	.....Slate right ear and left eye and hip, yellow right shoulder and pleura
BrnRp	.....Black eyes, ears and red pleura
RBlw	.....Red and black mixed but with red on nose
RBBw	.....Red and black mixed but with black on nose
RBlw	.....Red and black mixed with the hair on the nose clipped
B	..... Black

It will be noted that the entire description of the animal is recorded without raising the pen or pencil from the paper.

Since this system is based upon abbreviations, the question may be asked, why not go still further and use shorthand characters? It is true that shorthand once learned would consume less time, but it would require considerable training for a new assistant to become sufficiently familiar with shorthand characters to write them rapidly and accurately. In the author's experience, on the other hand, it has not required more than two or three fifteen-minute periods to instruct any new assistant in the satisfactory use of the above method.

A NOTE ON THE PREPARATION OF AGAR AGAR CULTURE MEDIA

C. L. WILLIAMS AND H. P. LETTON
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One of the greatest troubles in preparing agar agar is clearing the medium. Filtering through paper is very slow and requires a special water jacket for the filter in order to keep the agar hot enough to prevent solidification. Nor does a paper filter entirely clear the agar. The more usual method of filtering through cotton requires repeated filtration, and even then seldom gives a perfectly clear product.

The principal cause of failure is apparently the presence of finely divided insoluble particles derived from the agar agar, which become evenly distributed through the medium after this substance has been dissolved. These particles are often so fine that a great part of them will pass even through filter paper.

In the laboratory of the Sanitary District of the Great Lakes various methods were tried to facilitate filtration while securing a clear product. The use of egg albumin as a coagulant in some media suggested coagulation as probably successful, and the use of the Hygienic Laboratory method of preparing Endo's medium gave us the clue to the present procedure.

In the Hygienic Laboratory method for Endo, the medium is partially cleared by pouring into large beakers while hot, where it is allowed to cool and harden. On inverting the beakers the solid mass of agar slips out, and the bottom where most of the suspended matter has settled is cut off and wasted. This process was modified by us in the case of ordinary agar media by placing the container in a large water bath (a large saucepan usually served for this) which was kept over a low flame for several hours, usually over night. This procedure caused the collection of the suspended matter into flocculent masses which settled to

the bottom. The result was more than a settling of the solid particles, it was rather the ensnaring of these in a delicate coagulum, much like the coagulum of aluminum hydrate and like that substance in requiring some time for its action. After the formation of this flocculent precipitate a single filtration through cotton produces a beautifully clear filtrate.

The following points should be remembered. Any stirring before filtration will quickly break up the coagulum, which is very delicate, a cloudy filtrate resulting. For this reason, the reaction should be adjusted before placing on the water bath, as we have found that this changes very little during its stay there. After sterilization in the autoclave the medium remains clear if soon cooled. If remelted for pouring plates it is clear at first, but after standing some time in the water bath a second flocculent precipitate forms, which however settles to the bottom so that with careful handling the clear supernatant medium may be readily poured off.

No attempt has been made by us to review the literature dealing with this subject, so that we do not know if this procedure has been described before. We have never seen it mentioned however. Any one who has had to make any considerable amount of agar will readily understand the value of so simple a method of obtaining a clear product. We prefer this method even to clearing with egg albumin, since the result is a clearer medium although it takes more time to prepare.

BOOK REVIEWS

A Text-book upon the Pathogenic Bacteria and Protozoa. For Students of Medicine and Physicians. By JOSEPH MCFARLAND, M. D. Eighth edition, thoroughly revised. Octavo of 807 pages with 323 illustrations, a number of them in colors. Philadelphia: W. B. Saunders Company. 1915. Cloth $4.00 net.

The author opens with an excellent historical introduction, tracing the evolution of bacteriology through three stages—biologic contributions, chemical contributions and the modern medical and surgical contributions. The first part of the book, headed "General," is devoted to a discussion of the biology of microörganisms, methods of studying them, and infection and immunity. Chapter I—on Structure and Classification of Microörganisms—includes bacteria, higher bacteria, yeasts, molds and protozoa. No mention is made of the chemical composition of bacteria. The nature of the cell substance is passed over in one sentence. The author does not take up the bioligoc significance of spores, the conditions under which sporulation takes place, and conditions under which germination occurs. The classification of bacteria is treated very briefly, Migula's tables being given, while Jensen's system does not appear. Only ten pages are given over to a discussion of these subjects, about one-third of space allotted in other books.

Chapter II (pages 50–65), on Biology of Microörganisms is too compact and synoptic in its introductory discussion of biologica relations of bacteria to temperature. Mere definitions of minimum, optimum and maximum temperatures ove no significance without some discussion of the thermal death points of various organisms, range of temperature at which growth takes palace among different types, etc. Similarly more than one page should be devoted to a discussion of the food supply of bacteria, and the synthesis affected by nitrifying bacteria should be included under such a topic

Chapters III and IV (pages 66–143) on Infection and Immunity are clearly presented. The Lateral Chain Theory of Ehrlich is explained by a lengthy quotation from his Croonian Lecture before the Royal Society of London. A section in this chapter is devoted to Defensive Germents with a description of the Abderhalden Reaction and the technique involved in making the test. Staining and observation of protozoa, and bacterio-vaccines and their preparation are some of the new topics treated.

Chapters XI, XII and XIII (pages 234–248) devoted to Bacteriology of Water, Air and Foods are necessarily brief and incomplete.

In Chapter XVII (pages 251–262) on Determination of the Value of Antiseptics, Germicides and Disinfectants, a detailed description of the technique of the Anderson-McClintic method is given. The important theoretical work of Chick is not mentioned in this chapter.

Part II is devoted to a discussion of the infectious diseases and the specific microörganisms—each disease being treated in a separate chapter,—and this Part is very fully and well treated.

It is curious that the importance of septic sore throat as a new disease should not find a place in a discussion of streptococci. Cole's work on the Pneumococci has not yet been included in the chapter on Pneumonia.

Chapters XIX (pages 471–493) on Malaria and Chapter XX (pages 494–505) on Sleeping Sickness treat these subjects in much detail.

There is no mention of Texas Tick fever or Rocky Mountain Spotted Fever. Such diseases as measles, scarlet fever, foot and mouth disease and mumps should find a place in a chapter on filterable viruses, which the book does not contain.

On the whole the text presents the subject very clearly, and the illustrations are exceedingly good. The author in the preface says that the book is a medical work intended for medical students and practitioners; and its purely medical aspects are excellently handled. It is unfortunate however, that medical students should not master the undelying fundamental biological principles of bacteriology in their widest aspects for these are of the utmost importance in their application to modern preventive medicine.

D. GREENBERG.

The Principles of Pathologic Histology. By FRANK B. MALLORY, M.D., Associate Professor of Pathology, Harvard Medical School and Pathologist to the Boston City Hospital. Octavo of 677 pages, with 497 figures containing 683 illustrations, 124 in colors. Philadelphia and London: W. B. Saunders Company, 1914. Cloth $5.50 net.

This interesting and useful book on pathologic histology reflects admirably the experience of its well-known author. It is confessedly individualistic and contains practically no direct reference to the work and opinions of others. The author is undoubtedly right in insisting that the study of the lesions themselves is the only proper source of a balanced judgment in reference to the terminal aspects of disease. It seems a pity, however, that on controversial points he has not seen fit to state both sides of the question and then give his own interpretation.

The book may be reviewed from the standpoint of pathology in the larger sense, and again from the standpoint of the morphological aspects of disease. From the latter viewpoint it is to be praised for the orderliness of presentation and the remarkable clearness and beauty of the numerous original illustrations. In the first part, dealing with General Pathologic Histology, the subjects of inflammation and the retro-

grade processes are treated in a masterly and deservedly extended fashion. The section on Tumors, comprising approximately one-third of the book, is a remarkable monograph which presents a summary of the author's own extensive material basis on which his mature and deservedly respected opinion is based. The treatment of this phase, important as it is from a diagnostic standpoint, seems disproportionate for the needs of beginning students of disease processes however valuable it may be for the specialist. The second part dealing with Special Pathologic Histology is on the other hand somewhat short for general needs, and lays unusual emphasis on those rarer lesions which have happened to attract the author.

From the larger standpoint of pathology as a study of the natural history of disease the book represents an unfortunately restricted attitude. In studying disease we are of course interested in studying the cause and progress as well as the result produced. In the infectious diseases of known causation the entire course of the process can be followed, particularly with the aid of animal experimentation. Much can be learned concerning the progress of a disease if an approximately complete set of lesions is gathered by the diligent collector at the autopsy table, provided always that the successive stages be subsequently fitted into their proper places. Much more rapidly and certainly can a chronological series be prepared by injecting the specific microörganism into a suitable animal. To Mallory "recourse to animal experimentation has often served to confuse a subject rather than to simplify and clear it up." Experimentation unfortunately requires a certain type of ingenuity and the power of inductive reasoning in addition to the deductive reasoning required by the collector. The author has correctly stated that from a study of lesions alone "we are in a position to read the process (of disease) backward with some degree of certainty." It is profitable that the life work of a group of individuals should center in pathology as viewed from this essentially Chinese angle, but it is inexcusable that the work of the growing majority who choose to follow pathology forward rather than attempt to read it backward should not at least be recognized.

This failure to recognize the essentially dynamic functional viewpoint of disease has led Mallory into numerous errors in treating of the infectious diseases. Bacterial causation he recognizes in so far as bacteria may be fixed and stained, but failure to utilize or even absorb the experimental viewpoint has led to numerous unlikely hypotheses. Practically all bacteria are stated to act by the production of a "toxin" of greater or less strength as judged by the response of fixed cells; antitoxins are asserted to be formed in typhoid fever and indeed they are said to be formed in the so-called "endothelial leucocytes." It is needless to state that there is no experimental evidence for a statement of this sort. The very name of "endothelial leucocytes," for the description of which Mallory is properly appreciated, is probably inexact if we are to accept their origin from connective tissue as shown by Evans in his work with vital stains. For his insistence on the objectivity of lesions we have only to thank Mallory, but it is unfortunate that he has not employed his imagination in experimental verification of his working hypotheses.

F. P. Gay.

ABSTRACTS OF AMERICAN BACTERIOLOGICAL LITERATURE

ANIMAL PATHOLOGY

Notes on the Histo-Pathology of the Intestines in Young Chicks Infected with Bacterium pullorum. G. EDWARD GAGE and JAMES F. MARTIN. (Jour. Med. Res., 1916, **34**, 149–155.)

Typical strains of *Bacterium pullorum*, when injected into young chicks, produced death with characteristic symptoms of bacillary white diarrhea in 49 per cent of the animals. Chicks which survived the injection showed retarded growth and general weakness. The chief pathologic changes on section were marked injury to the mucosa, associated with hyperemia, hemorrhagic exudation and leucocytic infiltration.

H. W. L.

The Diptheroid Bacillus of Preisd-Nocard from Equine, Bovine and Ovine Abscesses. Ulcerative Lymphangitis and Caseous Lymphadenitis. I. C. HALL and R. V. STONE. (Jour. Infect. Diseases, 1916, **18**, 195–208.)

The authors call attention to the presence in the United States of the bacillus of Preisz-Nocard, which they isolated from horses and from a calf. They point out the uncertain significance of experimental orchitis in guinea-pigs as a test for glanders, and emphasize the necessity of microscopic and cultural examination of pus for diagnostic purposes.—P. B. H.

BACTERIOLOGY OF SOILS

Studies in Sulfofication. P. E. BROWN and H. W. JOHNSON. (Soil Science, 1916, **1**, 339–362.)

It is suggested that applications of sulfur-containing commercial fertilizers to soils will prove profitable unless different methods of soil treatment are employed than those in use at present, because at some future time sulfur will be lacking. In other words, for permanent soil fertility, the sulfur supply for crops must be considered. Chemical tests were made for determining sulfofication produced in a sulfur-rich soil to which various chemicals were added.—Z. N.

Preliminary Experiments on Some Effects of Leaching on the Soil Flora. C. B. LIPMAN and L. W. FOWLER. (Soil Science, 1916, **1**, 291–297.)

It seems that leaching affects the bacterial flora of soils profoundly. Nitrification, nitrogen-fixation and cellulose decomposition are wholly

or almost wholly checked by this process, especially if salts are present prior to leaching. Investigations are being carried on to determine if the injury done is or is not an ephemeral one which may entirely disappear in a few months under field conditions. This and other experiments in progress may lead to the discovery of the cause or causes of damage done to relatively new soils by irrigation flooding.—Z. N.

Quantitative Method for the Estimation of Bacteria in Soils. R. C. Cook. (Soil Science, 1912, **1**, 153–163.)

A synthetic medium is most desirable so that results may be comparable at different times and places, and for quantitative work the medium which will permit the development of the maximum number of colonies is usually most satisfactory.

Twelve media were at first compared on four soils, Lipman and Brown's modified synthetic agar being taken as a basis for comparison, the only difference being in the methods of sterilization. In the third series of experiments seven media were eliminated and in the last two series, Lipman and Brown's agar was eliminated. Results indicate that sodium asparaginate agar, albumen agar, and urea ammonium nitrate agar will in most cases give a greater colony development for soil bacteria than other media in common use in bacteriological work. A five-day count gives much higher bacterial counts than a three-day count.—Z. N.

Stimulating Influence of Arsenic upon the Nitrogen-fixing Organisms of the Soil. J. E. Greaves. (J. Agr. Res., 1916, **6**, 389–416.)

The author has previously shown that arsenic stimulates the nitrifying and ammonifying powers of soil. The present investigation is to show whether it has a similar effect upon nitrogen-fixation. Results show that all forms of arsenic tested, except paris green, stimulate the nitrogen-fixing power of soil, but not so greatly as the nitrifying power. These results are obtained only when the tests are made in soil (either untreated soil or sterilized soil reinoculated); for when solutions adapted to nitrogen-fixation are inoculated with soil, the addition of arsenic always proves toxic to the organisms concerned. One culture of *Azotobacter* was obtained that was directly stimulated by the arsenic; but in general, increase in nitrogen-fixation is observed only when a mixed flora is used. If the soil infusion used for inoculating sterile soil is previously filtered or heated as high as 55°C., no stimulation is brought about by the arsenic.

The author does not favor any one theory to account for these results; but considers the suppression of harmful microörganisms to be at least a partial explanation. He points out that the results are almost exactly what might be expected on the assumption that these harmful organisms were protozoa.—H. J. C.

The Occurrence of Bacteria in Frozen Soil. E. C. HARDER. (Bot. Gaz., 1916, **61**, 507–517, two graphs.)

Investigations were begun during the latter part of October and the samples were examined every week until the latter part of February. A dark, medium rich, slightly sandy garden soil was studied, obtained from the University campus near the College of Agriculture at Madison, Wisconsin. The soil was sampled to a depth of 6 inches each time. The entire sample was thawed when necessary and thoroughly mixed in a mortar previously washed out with 95 per cent alcohol. After proper dilution, plates were poured and counted after an incubation period of eight days at 28°C. Heyden Nahrstoff agar was used as a culture medium.

In order to determine whether the high bacterial content of frozen soil was due to an actual increase or whether other factors brought about this phenomenon, two duplicate sets of potted soils were prepared. One set was kept at room temperature and the other set was placed outside, subject to atmospheric temperatures.

Summary of results. 1. It was found that the number of bacteria in surface soil increased markedly after heavy frost, and in general maintained a high average during the winter months. The increase and decrease, however, were found to bear a distinct relation to the moisture content.

2. The potted soils failed to show such marked increase in the bacterial count after frosts. On the contrary the enriched cultures showed a distinct retardation of bacterial growth when in a frozen condition.

3. The bacterial flora was much the same during the fall, winter and spring, with the exception that after heavy frosts the small transparent colonies characteristic of water and of deeper soils, formed a larger proportion of the growth on the plates.

From these results it seems reasonable to conclude that ordinary soil bacteria undoubtedly withstand cold to a marked degree, even temperatures as low as 40°C. or more below zero. The increase in numbers, however, seems to be due to mechanical transpiration of moisture coming up from below during heavy frost, and where such transpiration is not possible there is an actual retardation in growth as compared with that in unfrozen soils.—J. T. E.

Incubation Studies with Soil Fungi. S. A. WAKSMAN. (Soil Science, 1916, **1**, 275–285.)

Too little attention has been directed to the possible influence of fungus forms as one of the important factors in soil fertility.

The longer and more complex life cycle of the fungi no doubt makes their relation to the fertility of the soil different at successive stages of growth.

Bacteriological methods were pursued in the study of three organisms which represent three very important groups of soil organisms: *Mucor plumbeus, Penicillium sp.*, and *Monilia sitophila.*

At first the moisture relationship and incubation period were deter-

mined for these three molds after 6, 12 and 18 days upon a culture medium composed of gravelly loam plus dried blood and cotton-seed meal as ammoniates. From results of this preliminary experiment attempt was made to determine how the biological stage affected ammonification. The periods of most active ammonification correspond to those of active spore formation for the respective organisms and the smallest amount to the time preparatory to actual spore formation. The *Monilia* shows the largest ammonia accumulation within the first 3 or 4 days; the *Penicillium*, between 10 and 15 days and the *Mucor* between 6 and 10 days.

An analogy is found in the growth of legumes like the clovers. Nitrogen is fixed during the period of active growth of the plant, the fixation ceasing almost entirely when seed formation begins.—Z. N.

Diastase Activity and Invertase Activity of Bacteria. GEORGE P. KOCH. (Soil Science, 1916, **1**, 179–196.)

This paper is limited to the study of diastases and invertases produced by bacteria which are concerned in the production of nitrogen compounds for consumption by plants and in the decomposition of carbohydrates in the soil.

The author determined that sufficient diastases and invertases were secreted by bacteria so that they could be quantitatively determined and also that there was considerable variation in the enzyme (diastase and invertase) secretion by organisms developed in culture solutions of different composition. The enzyme secretion by bacteria at different periods varies from day to day under conditions otherwise the same and there seems to be no direct correlation between hydrolytic enzyme secretion by bacteria and their property of decomposing proteins. The enzyme activity of various organisms and their ability to decompose proteins vary greatly; there is also a variation in enzyme activity of different cultures of the same species. No correlation was found between the secretion of enzyme and the decomposition of proteins by bacteria, the property of the cultural solution to rotate the plane of polarized light, the percentages of reducing compounds, the formation of acid or the numbers of organisms.

The rotatory power of a solution may be increased as well as decreased by bacteria; they do not produce a surplus of reducing compounds. Bacteria also seem to have the property of causing a condition which will prevent starch hydrolysis and sucrose inversion. There may be a possible correlation between the protein decomposition determined as ammonia and the formation of acid.—Z. N.

Bacterial Numbers in Soils at Different Depths, and in Different Seasons of the Year, SELMAN A. WAKSMAN. (Soil Science, 1916, **1**, 363–380.)

Four soils were studied. Meadow soil gave the largest bacterial counts at a depth of 1 inch, the 1-inch layer of this soil being richer also in organic matter and nitrogen content than that of the garden and orchard soils. The forest soil, though showing a high carbon and

nitrogen content gave the lowest bacteria counts probably because of the high acidity and large amount of undecomposed matter. The numbers of bacteria in the soils studied were not governed either by the moisture content of the different soils, or by the nitrogen and carbon contents.

The greatest number of bacteria were found at a depth of 1 inch in the soils that are under shade the year around. The garden soil gave on the average the largest numbers 4 inches from the surface. The numbers of organisms decreased regularly from a depth of 1 inch down to a depth of 30 inches.

Three soils showed a regular decrease in the lime requirement down to 30 inches and in all soils this was accompanied with a more or less gradual decrease in the nitrogen and carbon content.

Frozen soil, though showing a high bacterial content, did not give the largest bacterial numbers found through the year.—Z. N.

The Inoculation and Incubation of Soil Fungi. NICHOLAS KOPELOFF. (Soil Science, 1916, **1**, 381–403.)

The fungi employed in these studies were isolated from soil on the College Farm. They were identified as *Rhizopus oryzae* (Wendt), *Zygorrhyncus vuilleminii* (Namyslowski), *Rhizopus nigricans* (Ehrenberg), *Penicillium sp. 10*. Spores of these fungi were obtained for use by growing them on Cook's No. II fungi medium. Suspensions of a known number of spores of each fungus were added to 100 gm. portions of soil of an optimum moisture content to which dried blood and cotton-seed meal were also added. Ammonia determinations were made at the end of 7 days.

An increase in the number of fungus spores inoculated into the soil was found to be related to a proportional increase in ammonia accumulation but this increase was not proportional beyond a certain limit.

Under the conditions of moisture and temperature employed, cotton-seed meal appears to be the more acceptable source of food for the organisms studied. A 7-day incubation period may be recommended as most desirable for the study of soil fungi other than those belonging to the Penicillium group.

A striking increase in ammonia production was observed to take place every *other* day (after the first five days). This leads to the conclusion that the production of ammonia is dependent on the metabolic processes of the fungus rather than the biological stage of spore production and germination—Z. N.

Studies on the Decomposition of Cellulose in Soils. I. G. MCBETH. (Soil Science, 1916, **1**, 437–486.)

Detailed directions are given for the preparation of cellulose for cellulose agar. The action of cellulose-dissolving organisms was studied not only on typical cellulose such as is found in filter paper or in cotton fiber but on the cellulose of plant tissues freed from encrusting substances. The method for preparation of this plant cellulose is given in detail.

The cellulose-dissolving bacteria isolated from soils by means of the cellulose agar plate method, have the power of dissolving the cellulose of alfalfa. Twenty-five species of cellulose-dissolving bacteria dissolved alfalfa cellulose as readily as that prepared from filter paper.

Thirty-six species of this type of bacteria have been isolated and their cultural and morphological characteristics are given in detail. Fifteen new species are included.

A provisional key for identifying and comparing species of bacteria which dissolve cellulose is appended.

Many of the data called for by the card of the Society of American Bacteriologists seem to have little significance in the separation of members of this group.

Filamentous fungi and actinomyces also unquestionably play an important rôle in the destruction of cellulose in nature.—Z. N.

Actinomyces of the Soil. SELMAN A. WAKSMAN and ROLAND E. CURTIS. (Soil Science, 1916, **1**, 99–135, 3 plates.)

Seven different types of soils from different localities and under differing climatic and cultural conditions were used. Brown's albumen agar, slightly modified, was used for isolation of the actinomyces from the soil and for counts. Each organism isolated was studied on Czapeck's solution, agar, potato, and on 15 per cent gelatin in distilled water.

All the actinomyces studied liquefy gelatin and they may be divided into two groups depending on their differences in color production, one browning the liquefied portion, the other remaining colorless. A characteristic of some species is the production of an aerial mycelium on gelatin.

The numbers of actinomyces decrease with soil depth, but their numbers, relative to those of bacteria and fungi, increase.

Thirty species of actinomyces are classified, and their morphology and cultural characteristics on the above media are considered in detail. A key is included facilitating their identification.

The average optimum, maximum and minimum temperatures are 30°C., 50°C. and 15°C. respectively. The actinomyces do not play any appreciable rôle in the soil as ammonifiers; they readily assimilate NO_2, NO_3, NH_3 and organic compounds of nitrogen and characteristically reduce nitrate to nitrite, but not to free nitrogen or NH_3.

Actinomyces are strong cellulose decomposers and this fact in combination with their weak ammonia production leads the author to think that the probable rôle of the organism in the fertility of the soil lies in the formation of humus. Therefore, in arid soils where cellulose destruction has been found to be extremely rapid, actinomyces should be expected in abundance.—Z. N.

Some Factors that Influence Nitrate Formation in Acid Soils. E. B. FRED and E. J. GRAUL. (Soil Science, 1916, **1**, 317–338, 1 pl.)

Acid soils do not possess a strain of nitrifying bacteria especially resistant to soil acidity. The nature of the compound to be nitrified

plays an important part. For example, in acid soils organic nitrogen nitrifies much more rapidly than nitrogen from ammonium sulphate; the reverse is true in non-acid soils.

In soil treated with calcium carbonate there is an enormous multiplication of the nitrate bacteria. At first, one or two weeks after treating, calcium carbonate stimulates nitrate formation, later the reverse is true. In the presence of organic nitrogenous substances as casein and gelatin, calcium carbonate did not permanently increase the accumulation of nitrates.

All soils stored under conditions that prevent leaching showed a gain in nitrate nitrogen.

Considering the data given, as a whole, it seems that under laboratory conditions the beneficial effect of $CaCO_3$ on plant growth must be accounted for by some processes other than the direct effect on nitrification as this effect of $CaCO_3$ on nitrification takes place before higher plants begin to draw heavily on the nitrogen of nitrates. Moreover, the period of rapid accumulation from liming may result in a loss of nitrogen from leaching of the nitrates. These questions can be determined only by field tests.—Z. N.

The Influence of Some Common Humus-Forming Materials of Narrow and of Wide Nitrogen-Carbon Ratio on Bacterial Activities. P. E. Brown and F. E. Allison. (Soil Science, 1916, **1**, 49–75.)

Ammonification, nitrification and azofication were found to be increased to a considerable extent by application of the common humus-forming materials (dry) in maximum amounts for farm conditions; horse-, cow- and rotted manures, oat straw, timothy-, cowpea- and clover hays and corn stover. In general the manures favored and the legume hays depressed ammonification. Increases in ammonification were independent of the N-C ratio of the materials added, and were probably dependent on the chemical composition of the substances. If the humus-forming materials especially the manures, had been applied undried as under field conditions, ammonification would have been accentuated.

In contrast, nitrification was increased by leguminous green manures, and retarded somewhat by animal manures. These increases were apparently independent of the N-C ratio in the substances.

Azofication was favored by manure, straw stover and non-leguminous hays, and leguminous hays in order, the N-C ratio of these materials being of little or no significance as indicated by their effects on azofication.

Indications were, however, that non-legumes and straws might increase azofication on soils to a large enough extent to make their use more profitable than that of legumes which add nitrogen to the soil, but are somewhat more expensive to use.

The substances with wide N-C ratio decreased the crop yield while those of narrow ratios gave increases. The N-C ratio was found to be of more importance in determining the effect on the second crop of oats than on the first crop.

To increase azofication, non-leguminous manures must remain longer in the soil than leguminous in order to give time for considerable decomposition to occur before a crop is grown to test the effects.—Z. N.

Can Soil Be Sterilized without Radical Alteration? DAVID A. COLEMAN, H. CLAY LINT, and NICHOLAS KOPELOFF. (Soil Science, 1916, **1**, 259–274.)

An effort was made to devise some method whereby soil might be rendered sterile with a minimum amount of alteration. Four different lines of experimentation were carried on: 1. The intermittent sterilization of soil by dry heat; 2. Various chemical substances used as soil antiseptics; 3. Volatile antiseptics applied in partial vacuum; and 4. Volatile antiseptics applied under pressure at 80°C.

Both moist and dry soils were sterilized at 82°C. for 1 hour on 1 to 5 successive days. Ammonia determinations were made after each heating, also bacterial counts on Lipman and Brown's synthetic agar. All protozoa were killed at the first heating; species of *Penicillium* and *Mucor* persisted throughout. The numbers of bacteria in the moist soil decreased from 47,750,000 per gram on the first day to 1,500 on the last day while although there was an initial depression in numbers of bacteria in the dry soil, an increase occurred on the first day, then a gradual decrease only. This method of soil sterilization is decidedly more efficacious in the sterilization of moist than of air-dry soil. It increases the total solids in the soil about 46 per cent, which is only one sixteenth as much as by the common method of steam sterilization. Where the time element is of considerable importance this method is undesirable.

As soil antiseptics, were used 1 per cent (on the basis of 100 gm. of air-dry soil) of ethyl alcohol, ethyl ether, toluene, carbon bisulfid, chloroform and hydrogen peroxid. Chloroform caused a decrease of 86 per cent of the original bacterial content and caused the least alteration in the chemical constitution of the soil; carbon bisulfid and toluene were next in order.

In addition to the first four volatile antiseptics named above, osmic acid was used in the third method of sterilization. The antiseptic vapor was allowed to remain in intimate contact with the soil for 1½ hours for 3 successive days. Carbon bisulfid, toluene and ethyl alcohol, in order, caused a decrease of 99+ per cent of the original soil flora, although their action is more efficient in air-dry soil.

Carbon tetrachloride, carbon bisulfid, ethyl ether and chloroform were used in the heat plus pressure method. They were used at a temperature of 80°C. for 3 successive days upon moist and air-dry soil. The decrease in bacterial flora, in general, approximated 98 per cent. Carbon bisulfid was the only chemical which proved superior to the check treatment; it developed a pressure of 20 pounds. Ethyl ether which was least efficient developed only 6 pounds. A possible correlation might be obtained between the pressure developed during treatment and the effectiveness of the sterilizing agent. Carbon tetrachloride, which developed 5 pounds pressure is an exception.—Z. N.

Studies on Soil Protozoa. SELMAN A. WAKSMAN. (Soil Science, 1916, **1**, 135–153.)

The author found that no flagellates could be found in a living condition before sixteen minutes and no ciliates until after sixty-two minutes in a soil which was covered with water during these periods. The longer time allows a closer observation among the soil particles and a more thorough examination of the field. There is no doubt that there is a minimum moisture content for each soil below which protozoa cannot be found in a living condition.

Four soils were selected which varied in humus content and were under crops or orchard. Examinations were made every day for ten days in succession during which period several rains occurred. The moisture content proved to be a limiting factor but not the only one as the structure and humus content of the soil likewise play an important part. The protozoa found were all flagellates.

Another experiment with three different soils under different moisture conditions, part sterilized and part unsterilized, the latter plus dried blood, to each of which cultures of protozoa were added, brings out the fact that the sterilization of soil and addition of easily soluble organic matter will make the conditions optimum for protozoan activities at a lower moisture content than in corresponding unsterilized or untreated soils.

Protozoa (flagellates) found in the first inch of three cultivated soils varied in numbers from 1000 to 10,000 per gram of soil; at four inches, from 100 to 5000; at eight inches from 100 to 5000; at twelve inches from 10 to 100. None were found at a depth of twenty or thirty inches. The fourth soil, an acid forest soil, contained from 10 to 100 flagellates at a depth of one inch and at four inches; at eight inches only 1 to 10 were found and below that none. Ciliates and amoebae were not recorded as they occurred only occasionally.

Flagellates are present in greatest numbers at a depth of 1 inch where conditions favor microörganic activities, ciliates and large flagellates in largest numbers at a depth of 4 inches where the moisture conditions may be more favorable for their development. A list of the common types is given.

The flagellates are at once the most common and the largest group of soil protozoa.

Ammonification by bacteria is not influenced appreciably by soil protozoa although their presence acts detrimentally upon bacterial numbers.

This might be explained by one of the following assumptions: (1) if the protozoa destroy bacteria, they destroy non-ammonifying organisms; 2) the protozoa themselves take part in the process of ammonification; (3) the disintegration of the bacterial cells results in decomposition products which might be responsible for high ammonia production.—Z. N.

A Detailed Study of Effects of Climate on Important Properties of Soils. C. B. LIPMAN and D. D. WAYNICK. (Soil Science, 1916, **1**, 5–48, 5 plates.)

A soil block, 5 feet square and 3 feet deep from the fields of each of three state experiment stations (Maryland, Kansas and California) was moved to the two other experiment stations and placed in position as nearly as possible in the original order of layers. A similar block of soil was dug up and replaced in its position at every station. Strips 5 feet wide of untouched field soil surrounded the board frame of every plot which was placed in position. It was then possible to study at Maryland, at Kansas and at California four soil blocks as follows: First, natural field soil *undisturbed;* second, natural field soil *disturbed* and replaced; third and fourth, soil blocks obtained respectively from each of the other two stations. The chief aim was to show how any given soil in its natural location compares, after seven years, with the same soil under foreign conditions. Unfortunately no studies except brief chemical studies, were made at the initiation of the soil exchange experiment.

Studies of the hygroscopic coefficient, the moisture equivalent, the wilting point, changes in color and colloidal nature were among the physical studies made. Among chemical studies, complete chemical analyses were made in accordance with the official method, also humus and humus nitrogen determinations, total nitrogen and soil water-extract studies. Among bacteriological studies were: Counts on albumen agar, ammonifying power for dried blood, nitrifying power for the soil's own nitrogen, for dried blood, for cotton-seed meal, and for sulfate of ammonia, nitrogen fixing power in mannit solution, and qualitative tests for cellulose destruction.

The soils were described as follows: The California soil as "Sacramento silt loam," the Kansas soil as a "dark heavy loam," and the Maryland soil as a "light yellow clay."

It was found that soils change markedly in color in a period of seven years, and perhaps less, when moved to other climates. The differences are so great that samples of any one original soil from the three different stations today show no outward resemblance among themselves, but appear to represent three very distinct soil types.

In general the hygroscopic coefficient, the moisture equivalent, and the wilting point of any of the soils increased when the soil was placed at California. Some exceptions to this rule are noted.

Generally speaking bacterial numbers increase in arid soils placed under humid conditions. In general, also, the opposite is true for humid soils.

Ammonification, nitrification and nitrogen fixation follow the general trend of bacterial counts. In the case of nitrification, however, this applies to certain forms of nitrogen only. In the case of other forms of nitrogen very peculiar conditions exist which are fully explained in the text.

Cellulose destruction by soils proceeds with greater rapidity under arid than under humid conditions with any given soil type. Cellulose

destruction therefore appears to follow in general an opposite course to those of other microörganic activities in soils as affected by climate.

Marked changes in the acid soluble constituents of soils are wrought by climatic effects. The general tendency is for soils to increase in iron and decrease in alumina when placed under arid conditions, and vice versa.

Phenomenal losses in certain constituents in five years seem to have occurred in some soils even when the latter were not moved. Thus for example the Maryland soil lost in the period named enormous quantities of magnesia.

Large increases occur in the total water soluble constituents of California soil when it is moved to the Kansas or Maryland stations. On the other hand, the Maryland soil gains in water soluble matter when moved to Kansas or to California.—Z. N.

BACTERIOLOGY OF THE MOUTH

The Treatment of Pyorrhea Alveolaris and its Secondary Systemic Infections by Deep Muscular Injections of Mercruy. B. D. WRIGHT. (Medical Record, 1916, **89**, 807–811.)

Seventy-five cases of pyorrhea were treated with mercuric succinimide administered intramuscularly. In conjunction with the injections of mercury, surgical treatment of the gums and teeth was carried out.

In all cases, the pyorrhea was entirely cured. Secondary systemic infections, which were present in 54 per cent of the patients, were also cured in every case.

The author is convinced that mercury, because of its parasitotropic action, promises to be successful in treating various infections produced by the vegetable parasites.—M. W. C.

Constitutional Conditions Caused by Oral Sepsis. J. DALAND. (New York Med. Jour., 1916, **103**, 817–820.)

Foci of infection in the mouth may give rise to serious constitutional disturbances, such as endocarditis, acute parenchymatous nephritis, furunculosis, and particularly septic polyarthritis. Many other pathologic conditions may be traced to a septic mouth as the cause. The offending organism, in such cases, is usually a streptococcus. This bacterium, according to the work of Rosenow, may develop greater virulence than it possesses as it exists in the mouth, if it is transferred to other parts of the body where different conditions for growth are present.

As a direct causal relationship exists between oral sepsis and constitutional disease, the dentist should take particular pains to remove all manifestations of oral sepsis, and it should become a matter of routine procedure that any septic conditions of a patient's mouth should be reported to the physician.—M. W. C.

A Study of Endameba Buccalis in Alveolodental Pyorrhoea. F. M. JOHNS. (Am. Jour. Trop. Dis. and Prevent. Med., 1916, **7**, 372–376.)

The first part of this paper deals with a microscopic study of the lesion—demonstrating the presence of the *Endameba buccalis* in greatest numbers penetrating the healthy peridental tissue beyond the superficial zone of suppuration, and not living in the mass of bacteria, pus and semi-necrotic material filling the pocket.

Phagocytosis of bacteria is only noted in the few atypical forms that are swept into the pus of the pocket. From the depths of the lesion only a large nucleated cell is found to be constantly ingested.

Reproduction in the lesion is apparently only by bilateral fission. Cyst formation was not observed.

A significant fact was in the adhesion of numbers of bacteria to the ameba when observed in fresh and wet fixed preparations.

Endamebae following the injection of emetin are gradually decreased in numbers without any visible morphological or biological changes being produced. Forms in active reproduction are found up to the complete disappearance, the inference being drawn that the action of emetin upon pathogenic amebae is an indirect one in the dosage clinically possible.—F. M. J.

BACTERIOLOGY OF WATER AND SEWAGE

Bacterial Counts in Water Examination. J. RACE. (Amer. Jour. of Pub. Health., 1916, **6**, 488–496.)

The conclusions as given by the author are:

1. Not one of the media used at any one of the incubation periods or temperatures chosen gives a bacterial count that bears a constant ratio to the organisms of excremental origin as estimated by the *B. coli* test.
2. The blood heat count is the nearest to the *B. coli* content.
3. The largest count is obtained by the use of nutrient gelatin.
4. The bacterial count varies directly with the incubation period and inversely with the incubation temperature.
5. The largest count in the shortest incubation period is obtained at 27°C.
6. The ratio of the count at a given temperature to the count at a higher temperature decreases as the temperature of the water increases.

D. G.

Bacteria in Commercial Bottled Waters. MAUD MASON OBST. (Bulletin No. 369, U. S. Dept. of Agr.)

The paper is based on results obtained from the examinations in the Bureau of Chemistry during the last six years of from 1 to 17 samples of bottled waters from each of 110 American springs and from 57 sources in foreign countries.

The author concludes that the data as summarized show the need of improvement in the bacteriological condition of many of the brands of bottled water to be found in the market. There are some springs used for the production of commercial bottled waters which should not be used. In other cases, the contaminations found are clearly those of manipulation.

The results clearly show that bottled water can be made to conform to the requirements of the United States Public Health Service for drinking water furnished upon trains; that is, that not more than one 10 cc. sample out of five should show the presence of *B. coli*.—S. H. A.

Confirmatory Tests for B. coli in Routine Water Examinations. W. H. Frost. (Amer. Jour. of Pub. Health, 1916, **6**, 585–588.)

If no typical colonies develop within 24 hours on Endo plates made from fermentation tubes showing gas, further effort is made to recover *B. coli* as follows: (1) One or more colonies are transferred to lactose broth fermentation tubes. The formation of gas demonstrates presence of *B. coli*. (2) Plates are again made from the original fermentation tube. (3) A transplant is made from the original fermentation tube directly to another lactose broth tube. If steps (1) and (2) have both failed to recover *B. coli*, plates are are now made from this transplanted culture. (4) At the same time a transfer is made from this tube directly to a third fermentation tube.

If all of the above procedures fail to recover *B. coli* and gas is still formed in this fermentation tube, the inference is that gas in the preliminary test was due to an anaerobe.

The presumptive test for *B. coli* whether in lactose bile or lactose broth is subject to considerable variations in its specificity as indicating the demonstrable presence of *B. coli*. Careful confirmation of the presumptive test is especially important in the examination of treated waters, where the error in this test is greater than in raw waters.—D. G.

CLASSIFICATION OF BACTERIA

Studies on the Classification of the Colon-Typhoid Group. J. B. Thomas and E. A. Sandman. (Amer. Jour. of Pub. Health, 1916, **6**, 579–584.)

The conclusions as given by the authors are: Glycerine is of value in separating *B. cloacae* from *B. communior* and *B. aerogenes*, but is fermented by both of the latter types, while *B. communis* isolated from water frequently fails to ferment it.

Dulcite and adonite are of little value in separating *B. cloacae* from *B. aerogenes* and *B. communior* while a majority of *B. communis* isolated from water fail to ferment these substances.

Salicin is frequently fermented by all the lactose-positive organisms.

Raffinose is frequently fermented and dextrin usually fermented by all of the lactose-positive organisms.—D. G.

A Study on the Grouping of Meningococcus Strains. MIRIAM P. OLMSTEAD, PHOEBE L. DUBOIS, JOSEPHINE B. NEAL, and ROSE SCHWEITZER. (Journal of Immunology, 1916, **1**, 307.)

Twenty-nine strains of meningococci were studied as to their immunity reactions by the complement fixation method. Immune sera were prepared in rabbits with each of these strains, and cross fixations were carried out with extracts of each strain of cocci. Fourteen strains gave cross fixation and fell into one group. Eight other strains gave cross fixation and so fell into a second group. Three strains gave cross fixation with certain members of group 1 but not with all, and two strains were entirely heterogeneous. The organisms of the second group gave cross fixation with one strain of Dopter's parameningococcus but not with a second representative of his organisms.—J. G. H.

The Correlation of the Voges-Proskauer and Methyl Red Reaction in the Coli-Aerogenes Group of Bacteria. MAX LEVINE. (Jour. Infect. Diseases, 1916, **18**, 358–367.)

The author studied the reactions of 167 colon-like organisms from the horse, cow, pig, sheep, man, raw sewage and septic sewage. It was shown that the two reactions studied were correlated; further that (1) there was a better correlation between saccharose fermentation and source than between saccharose-dulcite fermentation and source; (2) cultures which formed acetyl-methyl-carbinol from glucose usually fermented salicin and glycerin, but dulcite only occasionally; (3) organisms that give the Voges-Proskauer reaction are rarely found in feces; (4) in this test the glucose-peptone-dipotassium phosphate medium of Clark and Lubs gives a more distinct test than glucose broth. Since this reaction is characteristic for non-fecal strains, the author concludes that it may be of considerable sanitary significance.—P. B. H.

DAIRY BACTERIOLOGY

Pasteurization as a Public Health Measure. C. M. HILLIARD. (Jour. Home Economics, 1916, **8**, 307–312.)

The article gives a comprehensive statement of the principles and methods of milk pasteurization, and discusses the relation of the practice to the public health, especially the health of infants. Bacterial reductions obtained, both quantitative and qualitative, are considered.—C. M. H.

Bacterial Testing Versus Dairy Inspection. C. E. NORTH. (Amer. Jour. of Pub. Health, 1916, **6**, 569–578.)

The author discusses the value of intelligent coöperation of laboratory worker and inspector. The function of the laboratory is to examine results and to locate insanitary milk. The inspector's function is to ascertain the causes and to apply the remedies. Simple laboratory equipment and methods within the reach of the small village are discussed.—D. G.

Some Observations on Causes of High Bacterial Counts in Milk. H. D. PEASE. (Amer. Jour. of Pub. Health, 1916, **6**, 563–568.)

In parts of the country where adequate field and laboratory investigations of milk supplies have been in operation high bacterial counts are most generally caused through inefficiently cleaned apparatus, or as a result of inefficient refrigeration. In locations where no supervision has been in operation and where the producers are more or less slovenly, high bacterial counts may be attributed to definite dirty conditions and are found even where the milk can be shown to have been delivered promptly and with the application of a reasonable degree of refrigeration.—D. G.

Colon Bacteria and Streptococci and Their Significance in Milk. L. A. ROGERS, W. M. CLARK, and A. C. EVANS. (Amer. Jour. Public Health, 1916, **6**, 374–380.)

The authors find that cultures (streptococci) isolated from infected udders are characterized by weak fermentation of the simpler sugars and an inability to ferment higher polysaccharides and alcohols, while cultures from bovine feces may be distinguished by ability to ferment polysaccharides and inability to attack alcohols. They believe that the presence in lactose bile inoculated with milk and incubated at 37°C. of streptococci forming distinct chains is good presumptive evidence of milk from infected udders.

Bacteria of the colon group occurring in market milk may be divided into two very distinct groups (1) that which agrees closely with the characteristic colon bacillus of the bovine intestine, yielding a low CO_2: H_2 ratio; (2) that which yields a high ratio; numerous in milk, but occurring very rarely in bovine intestine (1 out of 150 cultures).

The surface of dried grains is found to be source of a number of types of colon. That type occurring with greatest frequency probably corresponds to conception of *B. lactis aerogenes*, so that the ordinary presumptive tests and even the usual confirmatory tests are not necessarily proof of the contamination of the milk with fecal matter.—D. G.

DISINFECTION

The Antiseptic Action of Ether in Peritoneal Infections. J. SALIBA. (Jour. A. M. A., 1916, **66**, 1295–1297.)

The instillation of ether into the peritoneal cavity in infections by streptococci and *B. coli* served as a safe and beneficial antiseptic.—G. H. S.

The Bactericidal and Fungicidal Action of Copper Salts. L. M. DEWITT and HOPE SHERMAN. (Jour. Infect. Diseases, 1916, **18**, 368–382.)

The present study was initiated to ascertain (1) the killing power of the sulphate and chloride of copper and (2) their inhibiting action, on *B. coli, B. typhi, B. prodigiosus, B. tuberculosis* and *Staph. aureus;* also on species of yeast, *Aspergillus* and *Penicillium.* The results indi-

cated that copper is unreliable as a bactericide and as a fungicide, although some organisms are more susceptible than others. Long time experiments were more satisfactory than short time tests. It is said that solutions representing one part of the metal (2.5 parts of the chloride or 4 parts of the sulfate) to the million kill the ordinary water organisms *B. coli* and *B. typhi*, but do not injure the health. One part to 100,000 was found to inhibit the growth of *B. tuberculosis* in vitro. The therapeutic value of copper salts is discussed.—P. G. H.

IMMUNOLOGY

Immunity, Natural and Acquired. W. H. PORTER. (Medical Record, 1916, **89**, 983–987.)

A theory of immunity which bases the formation and activities of antibodies upon the metabolic processes of the body proteins.—M. W. C.

The Production of a Hyperimmune Serum for Infectious Abortion in Mares. E. S. GOOD and WALLACE V. SMITH. (Jour. Infectious Diseases, 1916, **18**, 347–401.)

The authors report on the production of a serum which protected rabbits from the lethal dose, but did not protect a mare from artificial infection. In a guinea pig the time for abortion was lengthened.—P. B. H.

Complement Fixation in Vaccinia and Variola. JOHN A. KOLMER. (Journal of Immunology, 1916, **1**, 59.)

The sera of vaccinated rabbits and of a few recently vaccinated human beings gave complement fixation with salt solution extracts of variola virus and vaccine virus. Positive reactions were also obtained in 9 out of 17 smallpox patients. Alcoholic extracts of the virus could not be used as antigens as they gave no reactions with these sera.—J. G. H.

Complement Fixation in Varicella. JOHN A. KOLMER. (Journal of Immunology, 1916, **1**, 51.)

Kolmer obtained weak complement fixations in 7 out of 24 cases of chicken pox, the antigen used being a saline solution of the contents of the varicella vesicles.—J. G. H.

The Agglutinability of Blood and Agar Strains of Typhoid Bacilli. C. G. BULL and I. W. PRITCHETT. (Jour. Exp. Med., 1916, **24**, 35–40.)

Cultivation on 10 per cent rabbit blood agar did not affect the agglutinability of fifty-seven strains of typhoid bacilli. The authors were unable to confirm the observations of Gay and Claypole on the variation in agglutinability caused by cultivating the typhoid bacillus on blood agar. A typhoid bacillus showing irregularity in fermentation, agglutination, and indol production is described.—B. W.

A Simplified Method of Producing a Potent Precipitin Serum. WALLACE V. SMITH. (Jour. Med. Res., 1916, **34**. 169–175.)

The author finds that the serum proteins precipitated from blood by means of one-third saturation with ammonium sulphate constitute a potent antigen for the preparation of precipitin serum in rabbits. The dried precipitate can be kept on hand over a long period without impairment.—H. W. L.

The Agglutination Reaction with Sera Derived from Human Cases of Leprosy and from the Experimental Animal upon Various Members of the Acid-Fast Group. W. H. HARRIS and J. A. LANFORD. (Jour. Med. Res., 1916, **34**, 157–167.)

Sera from 20 human cases of leprosy tested against several strains of *B. leprae*, human, bovine, and avian tubercle bacilli, and several of the non-pathogenic acid-fasts, failed to show any regularity or specificity. The same was true of experimental sera produced by injections of the various strains of leprosy bacilli into rabbits.—H. W. L.

Observations on the Typhoid Reaction. C. R. AUSTRIAN and A. L. BLOOMFIELD. (Archives of Internal Medicine, 1916, **17**, 663–669.)

By the typhoidin reaction individuals who had suffered from typhoid fever or received prophylactic treatment could not be differentiated from those who had never had the disease nor received vaccine treatment. The reaction was positive in a large percentage of persons who gave no history of typhoid fever or artificial immunization.—G. H. R.

Erysipelas Migrans and Multiple Abscesses in a Six Months' Old Infant Successfully Treated with Vaccines. L. FISCHER. (Medical Record, 1916, **89**, 734–735.)

Report of a case, which showed no response to treatment with leucodescent light, ichthyol, evaporating lotions, or magnesium sulphate, but in which decided improvement and finally complete recovery followed the administration of stock streptococcus and autogenous vaccines.—M. W. C.

Vaccine Treatment. LUDVIG HEKTOEN. (Jour. A. M. A., 1916, **66**, 1591–1594.)

A discussion of the theory and practise of vaccine therapy. Following accurate diagnostic procedure an autogenous vaccine would seem the logical method of treatment. Stock vaccines and phylacogens, which are not standardized and may not be specific, have a limited application.—G. H. S.

Is the Hyperleucocytosis Following the Injection of Typhoid Bacilli into Immunized Rabbits Specific? HELEN I. MCWILLIAMS. (Journal of Immunology, 1916, **1**, 159.)

The experiments of Gay and Claypole showing hyperleucocytosis in immune rabbits following the injection of typhoid bacilli, were reviewed,

with the special object of determining whether this reaction was specific. The leucocytosis which McWilliams obtained in immune rabbits was no greater than that which normal rabbits showed and the typhoid immune rabbits reacted as strongly to colon as to typhoid bacilli.—J. G. H.

Specific Therapy in Certain Acute Infectious Diseases. F. J. DEVER. (New York Med. Jour., 1916, **103**, 972–975.)

Treatment of infectious diseases by their specific immune sera holds more promise of success than an active immunization with either plain or sensitised vaccines. This is due to the fact that the active immunity obtained by vaccines is produced relatively slowly and is of questionable value in an acute infection, while a specific serum, on the other hand, supplies antibodies without putting any added strain upon the body cells.—M. W. C.

Intravenous Serobacterin Therapeutics. W. E. ROBERTSON. (New York Med. Journ., 1916, **103**, 777–780.)

In acute infectious diseases, such as typhoid fever and pneumonia serobacterins, when administered intravenously, shorten the incubation period, and produce a particularly striking change in the blood picture, the most noticeable feature of which is a pronounced leucocytosis.

In local and chronic diseases the value of the intravenous use of serobacterins, as compared with other bacterins, has not as yet been tested.—M. W. C.

The Value of Autoserum Injections in Skin Diseases. W. S. GOTTHEIL. (New York Med. Jour., 1916, **103**, 1209–1211.)

Autoserum treatment in skin diseases does not itself effect a cure, but used in conjunction with local treatment it shortens the period of disease from weeks to days and lessens the probability of relapse.

Autoserum treatment is particularly useful in psoriasis. It is of frequent benefit in chronic urticaria, neurodermatitis, pruritus senilis and other itchy dermatoses; of some value in pustular acne and chronic eczema; and of no use in furunculosis, folliculitis, and other pus infections as well as pemphigus, lepra, lichen planus and syphilis.—M. W. C.

Autosensitized Vaccines. M. G. WOHL. (Medical Record, 1916, **89**, 770–772.)

Serobacterins which are sensitised with the patient's serum are more efficient than vaccines sensitised with a heterologous serum. This is due to the fact that the antibodies in the sera of the lower animals are not identical with those of human serum, and therefore cannot act as effectively as those found in the patient's serum.

An additional reason why autosensitised vaccines are superior is that the bacteria do not need to be freed from the immune serum by washing, but may be injected with the serum used in sensitising.—M. W. C.

Gonococcus-Complement Fixation: A New Lipoid Antigen. C. C. WARDEN and L. E. SCHMIDT. (Jour. of Lab. and Clin. Med., 1916, **1**, 333–347.)

In complement fixation tests for gonorrhea, an antigen composed of an alcoholic solution of the fats of the gonococcus gave a much higher percent of positive reactions than a commercial watery antigen (Parke, Davis and Company). With the Warden antigen the positive reactions appeared earlier, persisted longer, and occurred in a larger number of doubtful cases. Every case which gave a positive reaction with commercial antigen was positive with the Warden antigen and usually to a much greater degree.—M. W. C.

The Bio-Chemistry of the Gonococcus in its Relation to Immunity. CARL C. WARDEN. (The Urologic and Cutaneous Review, 1916, **20**, 181–182.)

On analysis Gonococcus substance shows 12 per cent nitrogen, 20 per cent fat, together with phosphorus, sulphur, salts and ash. Washing removes nitrogen and fats. Gonococci possess at least three enzymes, one proteolytic, one hydrolytic and one a lipase.

An aqueous or normal salt suspension of gonococci behaves, physically, as a colloid with an electronegative sign and is flocculated or agglutinated by electrolytes of opposite sign and by other colloids such as serum, under certain conditions.—C. P. B.

The Complement Deviation Reaction Applied to the Diagnosis of Moniliasis of the Digestive Tract. I. G. MARTINEZ. (Am. Jour. Trop. Dis. and Prevent. Med., 1916, **7**, 390–391.)

An extract of 10 cc. normal saline extract of three agar tubes of different strains of monilias was made from seventy-two hours growth on Sabourand's glucose agar. These were shaken for two hours, 0.5 per cent carbolic acid added, and the extract heated for one hour at 56°C. This antigen was titrated according to the usual method, and 0.4 cc. found to constitute one unit.

In use, following the original Wassermann technic for complement deviation reaction, two cases of sprue and one suspected case gave a positive reaction. All other reactions with other sera were negative. —F. M. J.

Variations in the Pneumococcus Induced by Growth in Immune Serum. L. M. STRYKER. (Jour. Exp. Med., 1916, **24**, 49–68.)

Various strains of highly virulent pneumococci of Types I and II were grown and subcultured for successive generations in media containing homologous immune serums. The author found that the serum treated pneumococci became less specifically agglutinable, they could no longer absorb the agglutinins for normal strains, and, when injected into the animal body, failed to produce agglutinating sera for normal strains. Further, the virulence of the various treated strains was decreased, which may be due to the absence of capsules on such strains and

also to the fact that the organisms so grown are phagocyted in normal serum. It is interesting to note that the variations in pneumococci produced by treatment with immune serum do not persist after animal passage. Reversion to the normal type takes place readily.—B. W.

Further Observations on the Agglutination of Bacteria in Vivo. C. G. BULL. (Jour. Exp. Med., 1916, **24**, 25–34.)

Pneumococci, dysentery bacilli of the Shiga type, and *Bacillus mucosus-capsulatus* are agglutinated immediately when injected into the circulation of actively immunized rabbits. *Staphylococcus aureus* and *albus*, colon bacilli, meningococci, gonococci, and non-virulent pneumococci agglutinate in the circulation of normal rabbits. Bouillon cultures of *Bacillus avisepticus* are highly toxic for both rabbits and dogs. The fresh sera of these animals have no bactericidal action upon the bacteria. Dog serum opsonizes the bacilli *in vitro*, and they are agglutinated and opsonized in the circulation and organs of normal dogs. On the other hand, this does not occur in connection with normal rabbits. A very small quantity of culture produces a fatal septicemia in rabbits but a subtoxic dose is without effect in dogs. The degree of agglutination and opsonization of bacteria within the animal body is inversely parallel to the infectiousness of the bacteria for the host.—B. W.

A Simple Method of Quantitative Determination of Complement Fixation. J. O. HIRSCHFELDER. (Jour. A. M. A., 1916, **66**, 1386–1387.)

The author gives his technic for performing complement fixation tests.—G. H. W.

The "Delayed Negative" Wassermann Reaction. O. M. OLSON. (Jour. Lab. and Clin. Ned., 1916, **1**, 704–705.)

The "delayed negative" reaction differs from the original Wassermann only in the method of reading the test. Instead of one reading at the end of two hours, readings are taken at twenty minute intervals while the tubes are in the incubator.

By this method the progress of hemolysis may be observed, and the relation of the time of reaction between the positive and negative controls and the serum under test may be noted.

Whenever hemolysis begins later and is slower in producing complete lysis than the negative control, the serum under test is called a "delayed negative" and is considered indicative of syphilis.—M. W. C.

The Antagonistic Action of Negative Sera upon the Wasserman Reaction. A. W. SELLARDS and G. R. MINOT. (Jour. Med. Res., 1916, **34**, 131–147.)

The authors report observations on the ability of normal negative sera to antagonize the reaction between a positive syphilitic serum and antigen. Working on the theory that, with a non-specific antigen such as is now commonly used for the Wassermann, binding may result from either an increase of specific complement-fixing antibodies, or a de-

crease in the normal antagonistic action, they were able to show that negative sera possessed, in varying degrees, substances which inhibited the binding power of positive sera. Of 119 cases tested, only six failed to show this antagonistic action. All negative sera were inactivated and none were used which contained two units or more of natural sheep hemolysin. Although of no practical application in routine Wassermanns, this factor may be of importance in complement fixation in other diseases.—H. W. L.

Further Observations on the Schick Test for Diphtheria Immunity. G. B. WEAVER and B. RAPPAPORT. (Jour. A. M. A., 1916, **66**, 1448–1450.)

General discussion of the subject with data upon the presence of the reaction in healthy adults, in cases of post diphtheritic paralysis, in scarlet fever patients, and in tonsillitis patients.

The authors employ toxin neutralized to excess with antitoxin as a control.—G. H. S.

The Schick Test, D. M. GRISWOLD. (Jour. Lab. and Clin. Med., 1916, **1**, 441–443.)

Beside providing a quick and easy method for determining individual susceptibility to diphtheria, the Schick test affords a means of determining the duration of the passive immunity conferred by injections of antitoxin. Of six convalescents tested every second day after they had recovered from diphtheria, the earliest recurrence of a positive Schick was 3 weeks after 20,000 units of antitoxin, the longest period of immunity, six weeks.—M. W. C.

The Pseudoreaction in the Schick Test and its Control. A. ZINGHER. (Jour. A. M. A., 1916, **66**, 1617–1618.)

The Schick test is due to the action of the soluble diphtheria toxin while the pseudoreaction is an anaphylactic response to the protein of the diphtheria bacillus.

To control such pseudoreactions an injection of diphtheria toxin heated to 75°C. for five minutes may be given. The soluble toxin is thus destroyed and any reaction following such an injection is due to the protein of the diphtheria bacillus.—G. H. S.

The Diphtheria Toxin Skin Reaction. H. KOPLIK and L. J. UNGER. (Jour. A. M. A., 1916, **66**, 1195–1196.)

A simplified method of performing the Schick test is described.

Instead of the diluted toxin, with the necessary sterile accessories, pipettes, syringes, etc. the authors employ undiluted toxin and an ordinary hypodermic needle.

The needle is dipped into the toxin and introduced intradermally. The advantages of this method, aside from its simplicity, are that undiluted toxin will retain its potency, traumatic pseudopositive reac-

tions are entirely eliminated, and anaphylactic pseudopositive reactions are largely eliminated.

As controlled by the Schick technic complete uniformity resulted, showing that the amount of toxin introduced was sufficiently accurate to secure reliable results.—G. H. S.

Diphtheria Immunity—Natural, Active and Passive. Its Determination by the Schick Test. W. H. PARK and A. ZINGHER. (Amer. Jour. of Pub. Health, 1916, **6**, 431–445.)

The test is of great reliability when properly made. In a certain proportion of individuals a pseudo-reaction is seen but this can usually be distinguished clinically from the true reaction. The test possesses great value in determining clinically the immunization of susceptible individuals, and in clearing up the diagnosis of clinically doubtful cases of diphtheria. The Schick reaction has added further proof to the clinical and experimental observations that very toxic cases of diphtheria do better when given an early intravenous injection of antitoxin than when it is administered in any other way. The results obtained with the test in families seem to indicate that besides infection with virulent diphtheria bacilli, other factors, possibly hereditary in nature are concerned in the production of natural immunity to diphtheria. The Schick reaction can be applied with advantage in testing the patients, resident staff and nurses of contagious disease hospitals. By its use a considerable saving can be effected in antitoxin during diphtheria outbreaks. A more widespread use of an active immunization with mixtures of diphtheria toxin and antitoxin would help to lessen the disease.—D. G.

Immunity in Tuberculosis. G. A. WEBB. (Jour. of Laboratory and Clinical Medicine, 1916, **1**, 414–427.)

A comprehensive résumé of work upon immunity in tuberculosis.

Attempts of the author to immunize guinea-pigs with living tubercle bacilli were successful when gradually increasing numbers of individuals of an old human culture were used, but unsuccessful with recently isolated cultures.

Experiments with monkeys inoculated with gradually increasing doses of a human culture produced in some animals a resistance to 10,000 times the lethal dose. Such a resistance could not be constantly produced, however. Lack of success with monkeys was probably due to excessive virulence of the culture.

Children inoculated five years ago with a culture of which the minimal lethal dose for a guinea-pig was 125 bacilli are still healthy and do not respond to the von Pirquet test.

The author is convinced that infection takes place, usually in childhood, with very small numbers of bacilli. If an immunity in childhood could be raised to even a slight degree, it would probably be possible to vaccinate successfully against tuberculosis.—M. W. C.

Immunity Factors in Pneumococcus Infection in the Dog. C. G. BULL. (Jour. Exp. Med., 1916, **24**, 7–24.)

Intravenous inoculations of from 1 to 3 cc. per kilo of body weight of a bouillon culture of virulent pneumococci produce septicemia and meningitis in dogs. The injected penumococci leave the circulation rapidly, but begin to reinvade the blood from twenty-four to forty-eight hours later. The septicemia reaches its climax between the fourth and fifth days and then abruptly declines, the blood becoming sterile within from one to three days after the height of the septicemia is reached. The initial disappearance of the pneumococci from the circulation has been found to be due to agglutination of the diplococci in the blood stream and accumulation of the clumps in the lungs, liver, spleen, etc. If the dogs are reinoculated during the ascension of the septicemia, the injected diplococci leave the circulation as rapidly as in normal dogs. Cultures isolated in this stage of the infection, both before and from three to four hours after the reinoculation, are resistant to the agglutinins and opsonins of immune sera that agglutinate and opsonize the cultures with which the dogs were originally infected. Thus it follows that the pneumococci are able to reinvade the circulation because they have acquired a fastness to the existing antibodies and not because the antibodies have been exhausted. By reinoculating dogs at the time of the crisis in the septicemia it has been shown that the agglutination of the pneumococci is more rapid and complete and that the diplococci leave the circulation much more rapidly than in normal dogs. Hence acquired antibodies are operative within the animals at this time although they cannot be demonstrated *in vitro* until from twenty-four to forty-eight hours later. Pneumococci isolated as the infection is subsiding are more susceptible to the action of immune sera than the original cultures injected. It is probable that all the dogs would have survived the infection if a meningitis had not developed. In the acutely fatal cases of meningitis few pneumococci are phagocyted, while in the milder and convalescent cases much phagocytosis occurs. It is suggested that the incubation period of infectious diseases is due to the fact that the infecting agents must become adapted to the adverse conditions encountered in the newly infected host before they can multiply sufficiently to produce the symptoms of disease. It is further suggested that epidemics may arise because the infectious agent is passed from person to person in the ascending stage of the disease and thus enters new hosts in a state of maximum resistance to the natural antibodies of such individuals. When early contacts are avoided, epidemics tend to subside because the infectious agent is weakened by the action of acquired antibodies during the period of convalescence.—B. W.

LABORATORY TECHNIQUE

Hydrochloric Acid as a Decolorizing Agent for the Tubercle Bacillus. R. A. KEILTY. (Jour. A. M. A., 1916, **66**, 1619–1620.)

The following technic is given: Make thin smears, fix with heat, and stain with cold carbolfuchsin for five minutes. Decolorize for thirty seconds or more with 30 per cent hydrochloric acid. Counterstain with Loeffler's methylene blue.—G. H. S.

A Device for Protection against the Tubercle Bacillus. R. A. KEILTY. (New York Med. Jour., 1916, **103**, 1074.)

The device described is used to prevent the scattering of tubercle bacilli from a platinum loop while it is being flamed.

The apparatus consists of a tube of Russian iron, so constructed that it surrounds the flame of a Bunsen burner, fitting the burner tightly at the bottom. The platinum loop may be plunged into the flame and any masses which jump are caught upon the sides or bottom of the tube.—M. W. C.

Stabilized Gentian Violet. W. D. STOVALL and M. S. NICHOLS. (Jour. A. M. A., 1916, **66**, 1620–1621.)

To prevent deterioration of the gentian violet used in Gram's stain the authors suggest a stain of the formula:

Anilin	28 cc.
Gentian violet	8 gm.
95 per cent alcohol	100 cc.
N Hydrochloric acid	5 cc.
Distilled water	qs. ad 1000 cc.

Dissolve gentian violet in the alcohol. Add hydrochloric acid to the anilin and dissolve in water to make 900 cc. Filter the aqueous solution and add to the alcoholic stain. Filter.—G. H. S.

The Production and Collection of B. coli in Quantity on Synthetic media. ROBERT BENGIS. (Jour. Infect. Diseases, 1916, **18**, 391–396.)

The best medium was composed of the following: 2.5 per cent agar, 1 per cent ammonium lactate, and 0.2 per cent disodium phosphate, with or without 1 per cent calcium carbonate. The absence of lactose increased the efficiency. Inoculation of specially constructed plates was accomplished by means of a De Vilbiss atomizer. The growth was scraped off with a safety blade and placed in 75 per cent cent alcohol.—P. B. H.

Production of Clear and Sterilized Anti-Hog-Cholera Serum. M. DORSET and R. R. HENRY. (J. Agr. Res., 1916, **6**, 333–338.)

The occasional presence of the foot-and-mouth virus in hog-cholera serum makes it necessary to devise some means of sterilizing it. Heating for thirty minutes at 60° kills the foot-and-mouth virus; but unless

the corpuscles are removed from the defibrinated hog-cholera-immune blood, the heat causes coagulation and destroys the commercial value of the serum. Complete removal of the corpuscles by centrifugalization alone has proved impractical; but if they are first agglutinated by the addition of extract of the common white navy bean, complete separation from the serum is quite easy. The serum thus prepared has no harmful properties due to the bean extract; and can be heated for thirty minutes at 60° without undergoing any change.—H. J. C.

PLANT PATHOLOGY

Crown gall Studies; Showing Changes in Plant Structure Due to a Changed Stimulus. (Preliminary paper.) ERWIN F. SMITH. (J. Agr. Res., 1916, **6**, 179–182, with six plates.)

This is a continuation of work on crown gall in which its similarity to human cancer is pointed out. This paper describes some new points in the pathology of the disease.—H. J. C.

Transmission and Control of Bacterial Wilt of Cucurbits. F. V. RAND and ELLA M. A. ENLOWS. (J. Agr. Res., 1916, **6**, 417–434.)

It was shown several years ago by Erwin Smith that this disease (due to *B. tracheiphilus*) was transmitted by the striped cucumber beetle (*Diabrotica vittata* Fab.). The present work confirms this conclusion, and shows that the principal summer carriers of the disease are all species of Diabrotica. There is no evidence of spread through the soil (unless the roots are injured) or by means of infected seed. The disease can be controlled by early treatment with Bordeaux and arsenate of lead. Experiments are now being carried on to learn how to control the beetles that spread the disease.—H. J. C.

Further Evidence that Crown Gall of Plants is Cancer. E. F. SMITH. (Science, 1916, **43**, 871–889.)

After a brief survey of the main sub-divisions of the forms of cancer and their outstanding characteristics, the author presents a powerful argument for the "parasitic origin and essential unity of the various forms of cancer occurring in men and animals." The evidence is essentially presumptive, being drawn from the striking resemblance between Crown Gall of plants, which the author has proved to be caused by the *Bacterium tumefaciens*, and human cancer. The domination of the morphologists in cancer research is deplored and the attention of the experimental biologist and the bacteriologist is invited. So firm is the author's conviction of the parasitic nature of this malady that he exclaims: "I am now persuaded that the solution of the whole cancer problem lies in a study of these plant tumors." The discovery reported that the Crown Gall organism will produce teratoid tumors with regularity in various plants, not only in dormant buds but in the leaves, is a most astonishing and important contribution.—C. M. H.

Studies on the Crown Gall of Plants: Its Relation to Human Cancer. ERWIN F. SMITH. (Journal of Cancer Research, 1916, I, 231–258, 87 pl.)

The author reviews first the geographical and botanical distribution of crown gall. This disease is differentiated from hypertrophic enlargements in plants due to a few special parasitized cells as in the root nodules of legumes and slime mold infections in crucifers, as well as from granulomatous hyperplasia such as those of the olive tubercle where the bacteria are imbedded between the cells. Crown gall is a peculiar hyperplasia caused by *Bacterium tumefaciens* developing sparingly and only intracellularly, the parasitized cells being thereby caused to divide prematurely and repeatedly, which results in a great mass of non-capsulated small celled tumor tissue in which the bacteria themselves are invisible.

The tumor resembles cancer in many ways, notably in the exhibition of growth independently of function, vegetative activity being stimulated, functional activity depressed. The tumor can be stimulated by feeding or starved into quiescence. It can be grafted upon other plants of the same species. As in mammalian cancer, the nuclei of the tumor divide both mitotically and amitotically. Due to the rigid cell walls of plants, on the other hand, true metastasis seems not to occur.

The writer mentions especially the atypical arrangement of the tissues, their loss of polarity and the anaplasia, or undifferentiation of the cells. The production of tumors varying in structure according to the type of tissue experimentally invaded, among which were recognized embryonic organ inclusions analogous to the teratomata of mammals, is recorded for the first time.

The bacteriology of the parasite is discussed briefly, as are also some experiments with animals.—I. C. H.

PUBLIC HEALTH BACTERIOLOGY

Public Health Laboratories. R. G. PERKINS. (New York Med. Jour., 1916, **103**, 721–724.)

A discussion of the activities of public health laboratories. Among the suggestions made for furthering the development of these laboratories it is particularly recommended that there should be close association between the laboratory and a high grade university, in order that conditions should be made ideal for research work.—M. W. C.

The Microscopic Examination of Finger Nail Deposits. S. SCHNEIDER. (Jour. A. M. A., 1916. **66**, 1615–1617.)

The microscopic examination of finger nail deposits reveals the fact that the usual methods of cleansing the hands and nails does not result to any considerable degree in removing deposits.

Bacteriologic examination of deposits shows microorganisms to predominate in the following order: streptococci; staphylococci; *Bacillus coli;* bacilli, cocci, and spirillae derived from diverse sources; yeast

cells, spores and filaments of higher fungi; and larvae of vermes, amebas, diatoms, algae, etc.

Attention is called to the examination of such deposits from the medico-legal as well as from the hygienic standpoint.—G. H. S.

Controlling the Spread of Sputum. W. A. MANHEIMER. (Medical Record, 1916, **89**, 997–999.)

Sputum is one of the most dangerous of human discharges because of its wide dissemination and high content in pathogenic bacteria.

Experiments conducted to determine the viability of a culture of colon bacilli, when placed upon mailing envelopes as organisms in the sputum would be deposited in ordinary licking showed that of the bacteria sent through the mail, 5 per cent remained alive. Diphtheria bacilli placed upon envelopes and dried could not be cultivated in the few experiments performed for this purpose.

Valuable suggestions are given, which would aid in controlling the spread of sputum.—M. W. C.

MEDICAL BACTERIOLOGY

Case of Coccidioidal Granuloma. S. T. LIPSITZ, G. W. LAWSON, and E. M. FESSENDEN. (Jour. A. M. A., 1916, **66**, 1365–1367.)

Case report with detailed account of the blood picture and bacteriologic findings.—G. H. S.

The Contents of Ovarian Cysts. J. T. LEARY, H. J. HARTZ, and P. B. HAWK. (New York Med. Jour., 1916, **104**, 16–18.)

Bacteriological examination of the contents of six ovarian cysts resulted in negative findings in every case.—M. W. C.

Neisserian Proctorrhea. CHARLES C. MAPES. (The Urol. and Cut. Rev., 1916, **21**, 1.)

Infection of the rectal mucosa with the diplococcus of Neisser (*Gonococcus*) occurs more frequently than is generally believed.—C. P. B.

The Treatment of Human Rabies with Quinin and with Phenol. F. S. FIELDER. (Jour. A. M. A., 1916, **66**, 1300–1302.)

Several case reports of human rabies treated by injections of quinin or phenol. No specific action of the drugs was manifested.—G. H. S.

Trichinosis and the Cerebrospinal Fluid. W. LINTZ. (Jour. A. M. A., 1916, **66**, 1856.)

An accurate and rapid method of diagnosis in suspected trichinosis is to be found in an examination of the cerebrospinal fluid for *Trichina spiralis*.—G. H. S.

The Etiology of Typhus Fever in Mexico. (*Tabardillo.*) P. K. Olitsky, B. S. Denver, and C. E. Husk. (Jour. A. M. A., 1916, **66**, 1792–1692.)

An organism was isolated from typhus fever patients in Mexico which possessed morphological and cultural characteristics identical with those of the *Bacillus typhi-exanthematici.*—G. H. S.

The Etiology of Iritis. E. E. Irons and E. V. L. Brown. (Jour. A. M. A., 1916, **66**, 1840–1844.)

An etiologic study of 100 cases of iritis in which the causal relationships of syphilis, gonococcal infection, tuberculosis, dental, tonsillar, sinus, and genito-urinary infections are discussed.—G. H. S.

The Laboratory Examination of Material in a Case of Suspected Smallpox. J. N. Force. (Jour. A. M. A., 1916, **66**, 1384.)

Pus obtained from a case of suspected smallpox injected intradermally into immune rabbits, and controlled by injections of vaccine virus, demonstrated the absence of small pox. The test required but forty-eight hours.—G. H. S.

Experimental Studies in the Production of Chronic Gastric Ulcer. A. O. Wilensky and S. H. Geist. (Jour. A. M. A., 1916, **66**, 1382.)

Cultures of various strains of streptococci and yeasts derived from human gastric ulcers when injected into lesions artificially produced in the stomachs of cats failed to cause ulceration or retardation of healing.—G. H. S.

The Treatment of Genito-Urinary Tuberculosis with Rosenbach's Tuberculin. A. Hyman. (Jour. A. M. A., 1916, **66**, 1379–1381.)

Rosenbach's tuberculin, a product of the symbiotic growth of the tubercle bacillus and *Trichophyton holosericumalbum*, was used therapeutically in thirteen cases of urogenital tuberculosis. Two cases showed improvement, the remainder were not influenced.—G. H. S.

Recurrent Generalized Herpes of Infectious Origin. F. Cohen. (Jour. A. M. A., 1916, **66**, 1598–1599.)

Case report of generalized herpes simplex recurrent over a period of two years.

A streptococcus was isolated from the lesions and administered as a sensitized vaccine. Cure resulted.—G. H. S.

Streptothrix in Bronchopneumonia of Rats Similar to that in Rat-Bite Fever. R. Tunnicliff. (Jour. A. M. A., 1916, **66**, 1606.)

A streptothrix, apparently *Streptothrix murisratti* was isolated from several rats affected with bronchopneumonia. Morphological and cultural characteristics are given.—G. H. S.

Control of Diphtheria. D. M. Lewis. (Jour. A. M. A., 1916, **66**, 1535–1536.)

Emphasis is placed upon the value of epidemiological work in the control of diphtheria. It is asserted that the examination of the nasopharyngeal cavity of patients and contacts is more reliable than cultural work for the detection of carriers.—G. H. S.

Tuberculin in Surgical Tuberculosis. E. Bonime. (New York Med. Jour., 1916, **103**, 726–728.)

Tuberculin, if properly administered, is of great value in surgical tuberculosis. In cases where other infections occur in connection with the tuberculous processes, an autogenous vaccine should be used in addition to the tuberculin.—M. W. C.

Newer Laboratory Methods for the Early Diagnosis of Pulmonary Tuberculosis. M. H. Kahn. (Jour. of Lab. and Clin. Med., 1916, **1**, 599–607.)

A description of various laboratory tests used in the diagnosis of tuberculosis. The only test of absolute diagnostic importance is the bacteriological examination of the sputum. All others are as yet of but relative value.—M. W. C.

A Case of Infection of Lymph Glands with Bacillus Paratyphosus B. C. S. Cole. (Jour. Infect. Diseases, 1916, **18**, 349–352.)

A condition of multiple lymph-adenitis, first diagnosed as Hodgkins' disease, and later found to be a paratyphoid infection without the manifestation of typhoid-like, gastro-enteric symptoms.—P. B. H.

Etiology and Laboratory Diagnosis of Smallpox and Chickenpox. J. N. Force. (Jour. of Lab. and Clin. Med., 1916, **1**, 243–251.)

A review of the methods which have been proposed for the diagnosis of smallpox and chickenpox.

For absolute diagnosis or differential diagnosis between smallpox and chickenpox, the intradermal inoculation of the suspected material into vaccinia immune rabbits appears to be the simplest and most reliable method.—M. W. C.

The Immune Response in Pulmonary Tuberculosis. E. Bonime. (New York Med. Jour., 1916, **103**, 930.)

In Germany the early use of tuberculin in tuberculosis has caused a marked diminution in the death rate from the disease. Physicians of this country should be urged to combine tuberculin treatment with the usual hygienic measures employed in the early stages of tuberculosis.

M. W. C.

Flagellate Protozoa as an Etiologic Factor of Dysenteric Diarrhea. B. W. Rhamy and F. A. Metts. (Jour. A. M. A., 1916, **66**, 1190–1191.)

The authors assert that *Trichomonas intestinalis* is a cause of acute or chronic diarrhea following the drinking of impure water.

This conclusion is supported by case histories and by the report of an epidemic consisting of 78 cases with 17 deaths.

Ipecac and emetin proved valuable in treatment.—G. H. S.

The Causation and Treatment of Pellagra. H. E. Bond. (Medical Record, 1916, **89**, 816–819.)

The theory is advanced that pellagra is caused by bacteria occurring in the intestinal tract. Toxins produced act primarily upon the sympathetic nervous system and secondarily upon the central nervous system. Treatment should consist of the internal use of gastrointestinal antiseptics and the external use of protective ointments.—M. W. C.

Notes on Grip Epidemic in Chicago. A. M. Moody and J. A. Capps. (Jour. A. M. A., 1916, **66**, 1696.)

An analysis of 53 cases of grip shows that leucocytosis is usually absent. Bacteriologic examination of 31 cases gave the following results: *Streptococcus hemolyticus*, *Streptococcus viridans* and the pneumococcus were present 31 times; hemolytic staphylococci, 19 times; *Bacillus influenzae* and Friedländer's bacillus twice; *Streptococcus mucosus* and *Micrococcus catarrhalis* once.—G. H. S.

Antimeningococcic Serum in the Joint Manifestations of Gonorrhea. F. Malleterre. (New York Med. Jour., 1916, **103**, 1024–1026.)

Antimeningococcic serum is of value in generalized polyarticular forms of arthritis when several joints are involved and where there is moderate local inflammation without a fluid collection. The serum has little or no value in cases of gonorrheal monoarthritis with a large fluid collection.—M. W. C.

Pyelocystitis and Metastatic Abscesses Following Tonsillitis. H. B. Mills and G. A. Sowell. (New York Med. Jour., 1916, **103**, 725–726.)

Report of a case in which an attack of tonsillitis was followed by pyelocystitis and metastatic abscesses. *Staphylococcus albus* was the predominating organism isolated from the tonsils and was obtained in pure culture from the urine, blood and abscesses. The authors conclude that the tonsillitis was the etiological factor in the pyelocystitis, while the metastatic abscesses were complications of the latter.

M. W. C.

The Bacillus Epilepticus. C. A. L. Reed. (Jour. A. M. A., 1916, **66**, 1607–1611.)

The author asserts that epilepsy is due to infection by *Bacillus epilepticus*. The organism is a spore bearer, whose primary focus of infection is the cecum but which under suitable conditions may invade the circulation.

The bacillus has been cultured from the blood and digestive tracts of epileptics and has proved pathologic for rabbits.—G. H. S.

The Diagnosis of Enteric Fever (Typhoid and Paratyphoid A and B) by Agglutination Tests. W. C. DAVISON. (Jour. A. M. A., 1916, **66**, 1297.)

Macroscopic agglutination tests made in accordance with the quantitative technic of Dreyer are reliable as diagnostic procedures.

In cases of suspected typhoid in persons who have received prophylactic inoculation a series of tests made at intervals of 5 to 10 days are necessary to establish the diagnosis.—G. H. S.

Notes on the Etiology of the Recent Epidemic of Pseudo-Influenza. W. W. WILLIAMS and W. BURDICK. (Medical Record, 1916, **89**, 876-877.)

A streptococcus was isolated from cases of pseudo-influenza, which, immediately after isolation, was virulent for mice and rabbits. Autopsy of rabbits, killed forty-eight hours after inoculation with the strain, revealed a tracheal and bronchial inflammation, suggestive of a selective action. After four or five generations of subculture, however, no such selective action was manifest. Upon artificial cultivation, the organism also reverted to the usual cultural characteristics.

M. W. C.

Bacteriology in Conjunction with Homeopathic Prescribing. W. W. IRVING. (Jour. of Oph., Otol. and Laryng., 1916, **22**, 490.)

The author understands the action of the homoepathic remedy to be much the same as that of vaccines, in stimulating the body tissues to greater resistance. "Or does the remedy stimulate the anti body function or does it act as a germicide?"

By a study based on the above theories he expects to produce a materia medica which will unify the organism with the remedy.—C. P .B.

Anopheles Punctipennis, a Host of Tertian Malaria. W. V. KING. (Am. Jour. Trop. Dis. and Prevent. Med., 1916, **8**, 426–432.)

In two comparative experiments *Anopheles punctipennis* was proven to be an efficient host for the *Plasmodium vivax*. These mosquitoes were bred from the larvae and pupae collected in the open. They were fed individually on a gametocyte carrier. A high percentage of infection was obtained. Controls of *A. quadrimaculatus* were also found to be infected. The infection in all instances was followed to the salivary glands.—F. M. J.

Simultaneous Injections of Streptococci and Dahlia in the Guinea-pig. W. H. HOFFMAN, W. B. MACLURE, and L. W. SAUER. (Jour. Infect. Diseases, 1916, **18**, 353–357.)

From a study of the effects of combined injections the authors found that an injurious reaction resulted, although no change was detected in the opsonic content of the serum or in the hemolytic reactions in which the serum was employed. They conclude that "intravenous injection is not warranted as a therapeutic measure."—P. B. H.

The Site and Rate of Destruction of Pneumococci Following Intraperitoneal Injection. F. BERRY and C. O. MELICK. (Journal of Immunology, 1916, **1**, 119.)

This report confirms the observations of Kyes concerning the mode of destruction of pneumococci injected into an unsusceptible organism (pigeon). The pneumococci as in Kyes' experiments were found to be taken up in great numbers by endothelial cells in the liver, and spleen. They appear in these cells within ten minutes after intravenous injection and within about two hours after intraperitoneal injection, and disappear completely in from twenty-four to thirty-six hours.—J. G. H.

A Final Report on the Cultivation of the Tubercle Bacillus from the Sputum by the Method of Petroff. R. A. KEILTY. (Jour. Ex. Med., 1916. **24**, 41–48.)

Cultures of the tubercle bacillus were obtained in 12 out of 18 positives cases of pulmonary tuberculosis and of 7 negative cases 1 showed growth. From 4 of the 12 positive cases pure cultures were obtained. In 12 cases sterile cotton swabs were rubbed over the tonsils, fauces, tongue and gums, the swabs treated with 3 per cent sodium hydroxide and the neutralized sediment inoculated on the Petroff medium; and in one case acid fast bacilli were obtained.—B. W.

Post-Operative Tetanus. KELLOGG SPEED. (Surgery, Gyn. and Obstet., 1916, **22**, 443.)

A very complete résumé of the literature. In addition six cases are reported all occurring after some abdominal operation. The author believes that some human beings are tetanus carriers and when operation is done, any injury to the wall of the intestines may result in tetanus through invasion from the intestinal contents.

The possibility of haematogenous infection must also be considered; likewise external contamination from fecal discharges may carry the infection.—C. P. B.

The Bacteriology and Experimental Production of Ovaritis. E. C. ROSENOW and C. H. DAVIS. (Jour. A. M. A., 1916, **66**, 1175–1180.)

The authors prove experimentally that streptococci derived from inflammatory conditions of human ovaries show an elective affinity for the ovaries of animals.

Cultures from ovaries removed at operation yielded *Streptococcus viridans* in a large number of cases. The gonococcus, the Welch bacillus, *Staphylococcus albus*, the colon bacillus and diphtheroid-like bacilli were also obtained.

Microscopic preparations of sections of the ovaries showed the presence of diplococci in several instances.

Cultures of the streptococci isolated were injected into rabbits and dogs and were recovered in pure culture from the ovaries of the animals.—G. H. S.

Elective Localization in the Bronchial Musculature of Streptococci from the Sputum of Cases of Bronchial Asthma. S. OFTEDAL. (Jour. A. M. A., 1916, **66**, 1693–1694.)

Cultures from the sputum of asthmatic patients injected intravenously into rabbits caused marked interference with the respiration. The respiration was reduced in rate and labored. Microscopic examination of the lungs showed marked distention of the alveoli with frequent rupture of the alveolar walls. The blood vessels were engorged. The bronchioles were contracted. Hemorrhage and mud cell infiltration were present. Streptococci were found in the musculature.

Tissue cultures of the lungs yielded pure cultures of streptococci. The author considers this an example of the elective localization of streptococci.—G. H. S.

An Epidemic of Appendicitis and Parotitis Probably Due to Streptococci Contained in Dairy Products. E. C. ROSENOW and S. I. DUNLAP. (Jour. Infect. Diseases, 1916, **18**, 383–390.)

The authors studied an outbreak occurring in a military academy in which fifteen cases of appendicitis and thirty-four cases of parotitis arose between February and the following May. From the appendix and tonsils of the patients streptococci were isolated that were pathogenic for rabbits, reproducing lesions in the appendix. Strains isolated from dairy products at the time of the epidemic produced similar lesions in rabbits. The authors conclude that the epidemic was caused by infected dairy products.—P. B. H.

The Tuberculocidal Action of Arsenic Compounds and Their Distribution in the Tuberculous Organism. A. ARKIN and H. J. COOPER. (Jour. Infectious Diseases, 1916, **18**, 335–348.)

The authors studied the effect of arsenic upon the tubercle bacillus and its distribution in the body. They report that (1) Sodium arsenite in dilutions from 0.1 to 0.0001 per cent and sodium cacodylate in dilutions of from 2.0 to 0.002 per cent had no germicidal action in twenty-four hours at 37°C., although mercury cacodylate showed germicidal power. (2) Atoxyl, arsacetin and neosalvarsan in dilutions of 1.0 to 0.001 per cent showed no germicidal power. (3) These compounds were found in the liver, lungs, kidneys, blood, spleen and in the tuberculous tissues (lymph glands and eye). No evidence of accumulation in the tissues was obtained.—P. B. H.

Syphilis in Epilepsy. W. T. SHANAHAN, J. F. MUNSON, and A. L. SHAW. (New York Med. Jour., 1916, **103**, 820–824.)

While syphilis may be considered one of the many agencies producing epilepsy, there is no special type of syphilitic epilepsy.

The percentage of syphilis in epileptic patients is approximately the same as the percentage in the general population.

Treatment of syphilis in epilepsy does not usually result in improvement, probably because permanent injury has been done to the tissues long before the beginning of treatment.—M. W. C.

Focal Sepsis. J. DALAND. (New York Med. Jour., 1916, **103**, 1159–1160.)

Septic foci in the mouth, tonsils, sinuses, or prostate are frequently the cause of various systemic infections. The organism isolated from such foci is most frequently a streptococcus, usually *Streptococcus hemolyticus*. The severity of the systemic infection resulting from chronic focal sepsis varies with the virulence and number of the micro-organisms occurring in the focus, as well as with the resistance of the tissues to the spread of the infection.

The prompt diagnosis and removal of a septic focus is of the greatest importance.—M. W. C.

Meningitis. R. C. ROSENBERGER and D. J. BENTLEY. (New York Med. Jour., 1916, **103**, 1166.)

A report of seven cases, five of which were of the epidemic variety, one of pneumococcal, and one of tuberculous origin. The meningococcus was found in the spinal fluid of the five epidemic cases, and in one of the five a streptococcus was associated with the meningococcus. Cultures from a nasal discharge in two of the cases showed beside the usual bacterial flora, a gram negative diplococcus, which could not be differentiated from the meningococcus.

Antimeningococcus serum was administered to the five cases of the epidemic type. Three recovered, two did not.

Tubercle bacilli were demonstrable in the spinal fluid of the case with tuberculous meningitis two days before death. Pneumococci were present in the spinal fluid, and at autopsy, in the heart of the patient with pneumococcal meningitis.—M. W. C.

Tetanus: A Surgical Complication in the Present War. E. K. TULLIDGE. (New York Med. Jour., 1916, **103**, 1022–1024.)

The cases of tetanus treated during the present war, usually occurred as secondary infections, where the tetanus bacillus was found in wounded tissues in association with other organisms—most frequently with *Staphylococcus aureus* and *Bacillus aerogenes-capsulatus*.

The greatest mortality was in those cases of short incubation period, five days or less.

The most successful treatment was the administration of antitetanic serums in large doses, varying from 10,000 to 160,000 units.

Cases of long incubation period responded to smaller doses, but in cases displaying a short incubation period, large doses alone produce good results.

Chloral hydrate in doses of 5 to 10 grains was of great value in controlling convulsions. Cases where the wounds were treated locally with iodine did not develop the disease in its severest form.—M. W. C.

The Etiology of Common Colds. G. B. FOSTER. (Jour. A. M. A., 1916, **66**, 1180–1183.)

The conclusion that common colds are due to the action of a filterable virus is supported by the following observations:

The nasal secretions from individuals ill with colds were diluted with physiological salt solution and filtered through Berkefeld filters. The filtrates, when cultivated aerobically and anaerobically upon blood agar were sterile. Of ten men who were inoculated by placing some of the filtrate in the nostrils, nine developed colds.

The filtrates can be cultivated anaerobically in tissue—ascitic fluid. Stained preparations of the cultures were questionable but dark field examination showed the presence of active minute bodies possessing true motility.

Subcultures were prepared, filtered, and used to inoculate 11 men. After an incubation period of from eight to forty-eight hours all the men became ill with acute colds.

Filtrates from these experimentally produced colds could be cultivated.—G. H. S.

The Production of Amyloid Disease and Chronic Nephritis in Rabbits by Repeated Intravenous Injections of Living Colon Bacilli. C. H. BAILEY. (Jour. Exp. Med., 1916, **23**, 773–790.)

The repeated intravenous injection of rabbits with living *Bacillus communior* over long periods has resulted in the formation of amyloid deposits in the spleen, liver, and kidneys. Suppurative lesions were not present in most cases and therefore not a factor in their production. The results have been constant in that amyloid was found in all rabbits, eight in number, which were injected over a period of eighty-eight days or more. Eight rabbits showed amyloid in the spleen, six of these in the kidneys also, and three in the liver.

The kidneys of these eight rabbits also showed as a result of the injections a subacute and chronic glomerulitis, parenchymatous degeneration, some interstitial infiltration with round cells, and a slight cellular proliferation of connective tissue, thus resembling the chronic parenchymatous nephritis of man which is so commonly associated with amyloid disease.—B. W.

Anthrax with Report of Cases. S. J. ULLMAN. (Surgery, Gyn., and Obstet., 1916, **22**, 450.)

Reports two fatal cases of anthrax occurring in negroes who had helped to skin the carcass of a cow. The animals on this pasture were vaccinated against anthrax but one cow had escaped while being driven up. Several months later it was found dead on the range. Showing no lesion it was ordered to be skinned. Two days after this one of the negroes was taken sick; he died on the eighth day of the disease, no physician having been called. The other negro was taken sick a week later. He was treated with anthrax vaccine and with serum from a horse inoculated against anthrax. but died one week after symptoms developed.

Two other negroes who helped do the skinning were taken sick two weeks afterward. Serum was given immediately; they recovered.

The author is not sure that these two had anthrax but does believe a standard serum is of considerable value.—C. P. B.

An Epidemic of Dysentery at Fort Shafter, Hawaii, with Three Cases of the Hiss-Russell or "Y" Bacillus Infection. G. M. VAN POOLE. (The Military Surgeon, 1916, **38**, 525–530.)

An Epidemic of Bacillary Dysentery due to the Hiss-Russell Bacillus. M. A. DELANEY. (Ibid., 531–533.)

Bacillary Dysentery, Recent Epidemic at Fort Shafter, H. T., from the Laboratory Aspect. F. H. FOUCAR. (Ibid., 534–538.)

This epidemic of 34 cases began on October 16, 1915, and the last case was received November 21, 1915. During this time 3 officers, 2 children, 27 enlisted men and 1 civilian employee were stricken. Two deaths resulted, thus giving a mortality of 6 per cent. The first of these papers discusses the epidemic from the sanitary standpoint, the second from the clinical standpoint and the third from the laboratory standpoint, thus giving a comprehensive study of a sharp outbreak of bacillary dysentery which was soon brought under control. Perhaps the most interesting point brought out is found in Captain Foucar's paper which states that a vaccine was prepared from the "Y" Bacillus isolated from these cases. The vaccine was made in accordance with the technique used in preparing the army typhoid vaccine, but was used in smaller doses. Three doses at ten day intervals were given, mostly to children, a total of 168 separate doses being given. Only one severe reaction occurred, and it is stated that the cases among the children ceased although adult cases continued for some time.—E. B. V.

Study of a Strain of B. Welchii Isolated in France Together with Some Notes on Gastric Ulcers. MARY W. STEWART and RANDOLPH WEST. (Journal of Immunology, 1916, **1**, 189.)

Stewart and West studied an organism isolated from gas gangrene which was a strict anaerobe, was capsulated, non-motile, and formed spores only in sugar-free broth containing coagulated egg white. It produced stormy fermentation of milk and in other respects conformed with the *B. Welchii* type of the butyric acid forming group of bacteria. Weinberg and Sacquépée have reported the formation of a soluble toxin by a gas gangrene organism which was motile and which sporulated on sugar media but Stewart and West were unable to detect a soluble toxin in the cultures of their bacillus. The killed suspensions of washed bacteria were harmless for guinea pigs. The filtrate of sugar broth cultures which was highly acid was toxic to guinea pigs but this toxicity could be completely removed by neutralization of the filtrate. The most noticeable lesions produced by these filtrates were acute gastric ulcers and such ulcers could be almost as regularly produced by the injection of acetic acid solutions of similar titre. The necrotic tissue at the site of an intramuscular inoculation with the bacilli was also found to be highly acid. Blood cultures from infected pigs were rarely positive.

The conclusion is drawn that the general toxic effects in gas bacillus

infections are due not to generalized infection or to a true toxin, but to the toxic effect of the acid produced at the site of the localized lesion.
J. G. H.

Bacterial Cultures of Human Spleens Removed by Surgical Operation. ANDREW W. SELLARDS. (Journal of Immunology, 1916, **1**, 321.)

Eight spleens from cases of pernicious anemia, one showing simple hypertrophy, and one from infantile primary splenomegaly, all removed at operation, were the subject of this study. Aerobic and anaerobic cultures were made on various media including milk and glucose ascitic agar. From four of the spleens micrococci, differing from the ordinary pyogenic types but not further identified, were recovered. From three of the spleens, small Gram positive pleomorphic bacilli were recovered in the anaerobic milk tubes. These organisms culturally and morphologically resembled a culture of the bacillus recovered from typhus by Plotz. Complement fixation reactions were carried out with extracts of these bacilli against the blood of rabbits immunized to them. Cross fixation was obtained between the antigens from the two spleen cultures tested, with the serum of the animal immunized to the Plotz bacillus, and an antigen prepared from the Plotz bacillus gave fixation with the serum prepared by injection of the spleen cultures. Out of eight human sera tested, one from a case of pernicious anemia, and one from a case of cholangitis gave complete fixation with spleen bacillus antigen and with Plotz bacillus antigen. Other sera were negative. In fermentation tests, two of the spleen cultures differed from the bacillus of Plotz in fermenting mannite and in failing to ferment inulin, and one differed only in the absence of inulin fermentation. Sellards concludes that these organisms represent parasitic but non-pathogenic organisms found in the human body.—J. G. H.

Bacteriological and Experimental Studies on Gastric Ulcer. H. L. CELLER and W. THALHIMER. (Jour. Exp. Med., 1916, **23**, 791–812.)

Eight chronic gastric ulcers and one ulcer occurring at the ostium of a gastrojejunostomy were examined bacteriologically and histologically. From seven of these anhemolytic streptococci were isolated; streptococci were seen in cultures of the eighth but could not be isolated and from the ninth no streptococci were recovered. Yeasts were recovered from four. Other organisms including staphylococci, *Micrococcus tetragenus* and *B. subtilis* were isolated. The streptococci isolated were injected into rabbits and cats intravenously. Of thirty rabbits injected in the ear vein, four developed gastric lesions. Of eight rabbits injected in a branch of the gastric artery six developed gastric lesions. In two cats a branch of the gastric artery was injected with streptococci. Both animals developed defects in the gastric mucosa, which soon began to heal and were observed to have healed completely in thirty-three days. In addition fourteen of the thirty rabbits developed cardiac lesions, while two of the eight in which a branch of the gastric artery was injected developed minute hemorrhages in the endo-

cardium. The authors are unable to decide definitely whether or not the gastric lesions produced by the injection of the rabbits with streptococci are to be considered ulcers. From their experiments they conclude "It must be assumed that some cause is operative in certain cases preventing the healing of defects in the gastric mucosa and is inoperative in others. Even though anhemolytic streptococci are present in practically all gastric ulcers, we cannot convince ourselves that these organisms have been proven as yet to be the factor which either initiates the ulceration or prevents healing. Nevertheless, the constant presence of streptococci in this type of lesion is a suggestive fact and further experiments to determine their significance are being undertaken."—B. W.